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	45	1,100	751	972	1,173
1	15	900	1,118	526	657
2	30	1,000	1,113	573	649
3	45	1,100	704	908	601
5		1,350	521	1,183	281
6	30	1,275	647	737	111
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1. Bowman, H.W.: Clinical evaluation of dextran as a plasma volume expander. Paper read before the section of anesthesiology of the American Medical Association, New York, June 3, 1953.

2. Hyde, G.M., and others: The blood volume expansion produced by gelatin, serum albumin and plasma, Surg., Gynec. & Obst. 95:657 (Dec.) 1952.

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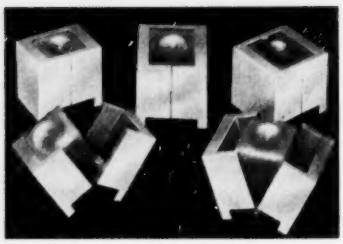

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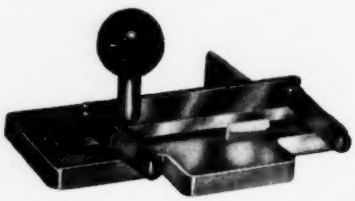

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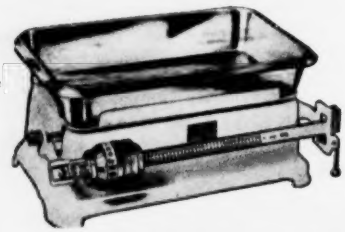

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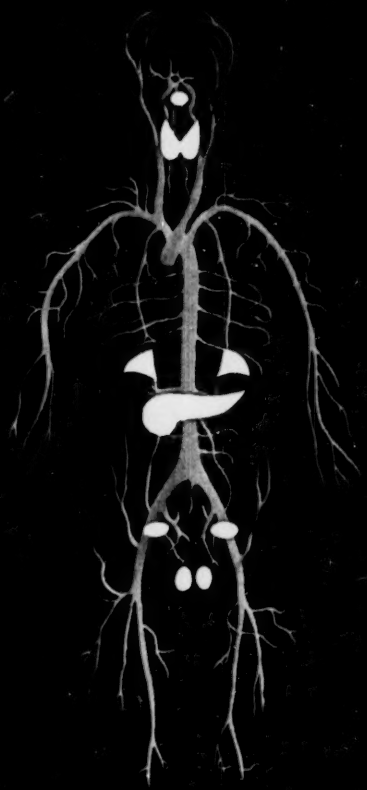

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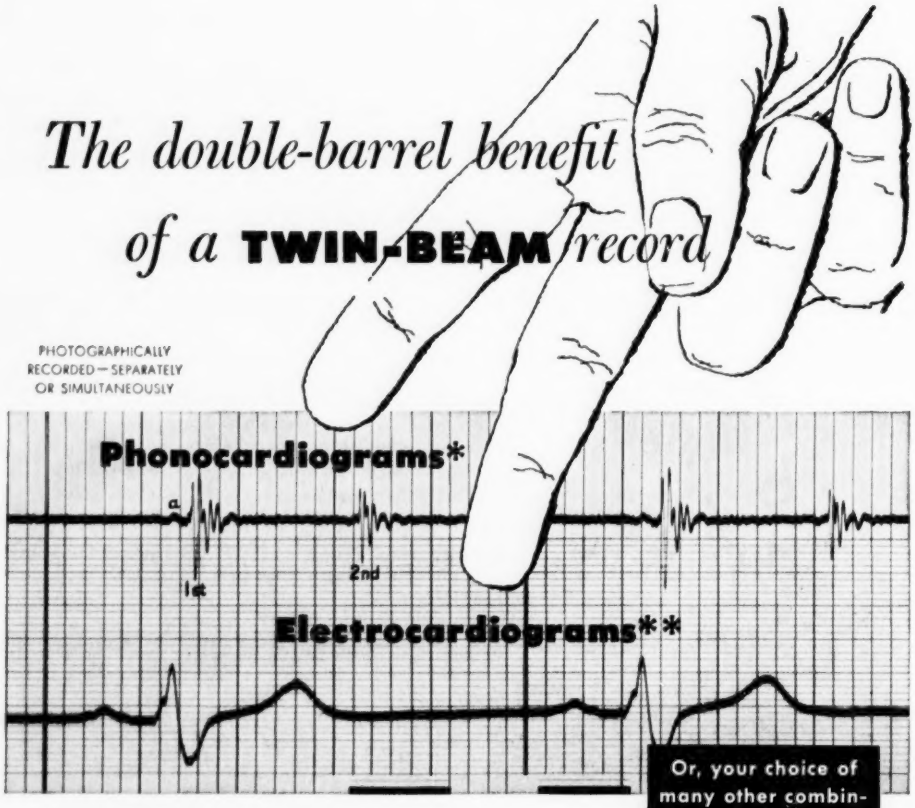

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THE EFFECT OF HEXAMETHONIUM BROMIDE ON SPLANCHNIC BLOOD FLOW, OXYGEN CONSUMPTION AND GLUCOSE OUTPUT IN MAN

By T. B. REYNOLDS,[1] A. PATON, M. FREEMAN,[2] F. HOWARD, AND SHEILA SHERLOCK

(From the Department of Medicine, Postgraduate Medical School of London, London, England)

(Submitted for publication April 2, 1953; accepted May 1, 1953)

The fall in blood pressure following autonomic blockade with methonium compounds could be attributed to a fall in cardiac output or to vasodilatation. In the recumbent position, however, cardiac output is usually well-maintained (1, 2, 3, 4) and blood flow through brain (5) and kidneys (1, 6) is not greatly altered. Increased blood flow has been reported in the digits and feet (1). This would hardly account for the large fall in blood pressure, and it seemed possible that the splanchnic area might be the site of the major vasodilatation. There is little information concerning splanchnic blood flow after hexamethonium. Freis and associates (1) state that it is not significantly changed, while Hoobler is said to have found a fall after tetraethylammonium chloride (7). We have therefore studied the effect of hexamethonium on splanchnic blood flow in subjects without liver disease. The opportunity has also been taken to make observations on the metabolic effects of hexamethonium on splanchnic oxygen consumption, hepatic glucose output and arterial lactic acid concentration.

METHODS AND MATERIAL

The 17 subjects had no known hepatic dysfunction or disturbance of carbohydrate metabolism, and had not previously received hexamethonium. Five had essential hypertension with diastolic blood pressures between 90 and 110 mm. Hg. Studies were made in the morning after an overnight fast, and one hour after an oral dose of 0.2 Gm. sodium amytal. A radio-opaque nylon catheter was introduced under fluoroscopic control into a radicle of the hepatic vein, and the subjects were then returned to a comfortable position in bed with the head on one small pillow. A priming dose of bromsulphalein (B.S.P.) was given and B.S.P. (4.5 to 7.0 mg. per min.) was infused at a constant rate. An in-dwelling needle was placed in a brachial or femoral artery. Thirty to forty minutes were allowed for stabilization; simultaneous hepatic vein and arterial samples were then taken for determination of B.S.P., glucose, lactic acid and oxygen. Three or four such determinations were made over twenty minutes and the results averaged to obtain the control values for splanchnic blood flow, hepatic glucose output, arterial lactic acid concentration and arterial-hepatic venous oxygen difference. The brachial blood pressure (auscultatory) and pulse rate were recorded frequently during this time. When the circulatory state was reasonably steady, hexamethonium bromide (1 mg. per Kg. body weight) was injected intramuscularly and the determinations repeated 5, 15, 30, 45 and 60 minutes later. In six subjects, observations were continued at 20 minute intervals for a further hour.

Estimated splanchnic blood flow (E.S.B.F.) was calculated with appropriate corrections for changing plasma B. S. P. levels (8). Plasma levels over 1 mg. per cent were maintained in all instances and extraction by the liver always exceeded 15 per cent. Plasma B.S.P. levels tended to rise after hexamethonium because of the marked fall in E.S.B.F. Decreasing the infusion rate by 20 to 30 per cent at the time of giving the hexamethonium usually prevented this rise. If the B.S.P. level five minutes after hexamethonium had risen in spite of this, the determination was discarded.

Hepatic glucose output (HGO) was determined by multiplying the arterial-hepatic vein glucose difference by the E.S.B.F. For the control value the average of the three or four arteriovenous glucose differences was multiplied by the average value for blood flow. Intestinal and splenic metabolism of glucose is probably negligible in the fasting state (9, 10) and the term "hepatic" rather than "splanchnic" glucose output has therefore been used throughout.

"Mean" blood pressure was expressed as half the sum of the systolic and diastolic auscultatory levels. In certain subjects this was checked by direct tracings obtained by strain gauge from the in-dwelling arterial needle.

Overall splanchnic vascular resistance was calculated by dividing the "mean" blood pressure (mm. Hg) by the splanchnic blood flow (ml. per min.) and expressed in arbitrary units.

"Portal venous pressure" was measured with a strain gauge in six subjects before and after hexamethonium by

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[2] Lund Research Fellow of the Diabetic Association (Great Britain)—to which we are also indebted for an expenses grant.

the technique of "occluded" hepatic vein catheterization (11, 12, 13), the catheter being advanced deep into the substance of the liver so as to occlude an hepatic vein. A close correlation has been found between the pressure recorded in this position and the portal venous pressure (13). These six subjects remained recumbent on the padded fluoroscopy table throughout the study.

Blood samples were preserved with heparin and sodium fluoride. Glucose was measured on duplicate 0.2 ml. whole blood samples (14), bromsulphalein on 1 or 2 ml. of plasma (15) and lactic acid on 2 ml. of whole blood (16). Oxygen unsaturation was determined without delay on 5 ml. blood samples collected under paraffin using the Haldane blood gas apparatus (17). Plasma volume (18) and surface area were calculated from tables.

RESULTS

Changes in Splanchnic Circulation (Table 1)

In 15 subjects the reaction to hexamethonium was similar (Figure 1).

The "mean" blood pressure fell within 5 minutes, and within 30 minutes had reached levels averaging 32 per cent below the control value. A fall in blood pressure occurred in all subjects, even though recumbent. The magnitude of the fall varied considerably, being greatest in those with the highest resting values.

Pulse rate increased by an average of 11 per cent of the resting value.

E.S.B.F. initially was slightly higher than previously reported values for normal subjects under similar conditions (15). After hexamethonium the E.S.B.F. fell, reaching an average of 67 per cent of the control value at the end of 30 minutes. The fall in E.S.B.F. paralleled the fall in "mean" blood pressure and the correlation coefficient, being more than six times its standard error, was therefore highly significant (Figure 2).

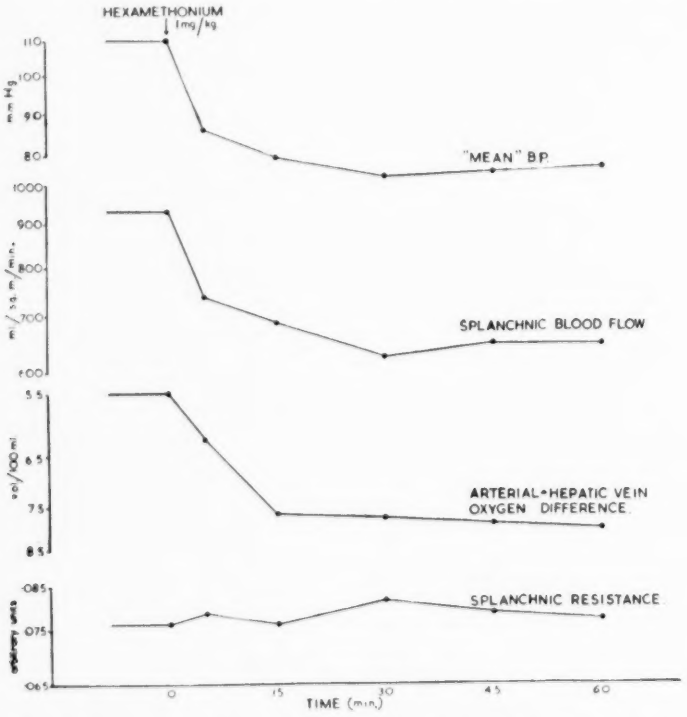

FIG. 1. MEAN RESULTS OF 15 OBSERVATIONS SHOWING EFFECT OF HEXAMETHONIUM ON "MEAN" B.P., SPLANCHNIC BLOOD FLOW (E.S.B.F.), ARTERIAL-HEPATIC VEIN OXYGEN DIFFERENCE AND SPLANCHNIC VASCULAR RESISTANCE

Semilogarithmic scale. The *increase* in arterial-hepatic vein oxygen difference is plotted downwards.

TABLE I

*Effect of hexamethonium on E.S.B.F., "mean" B.P., splanchnic vascular resistance and arterial-hepatic vein oxygen difference**

Subject	Age	Sex	E.S.B.F. (*ml. min./m²*) min. after C6						"Mean" B.P. (*mm. Hg*) min. after C6						Splanchnic Vasc. Resistance (*Arbitrary units*) min. after C6						Arterial-Hepatic Vein Oxygen Difference (*vol./100 ml.*) min. after C6					
			0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60
F. A.	35	M	940	960	880	950	1080	900	93	85	78	78	84	85	.054	.048	.048	.045	.042	.052	5.9	5.2	6.6	8.3	7.9	9.1
F. C.	42	M	1110	1070	800	810	—	820	90	83	91	87	86	88	.049	.046	.068	.065	—	.065	4.7	4.9	4.6	3.9	3.9	4.6
L. C.	46	F	720	400	430	350	490	520	125	90	68	63	61	60	.119	.155	.108	.123	.085	.079	5.3	6.4	8.4	7.8	8.3	7.1
E. C.	34	M	1070	—	1080	790	970	650	102	100	91	89	87	81	.059	—	.052	.069	.054	.075	—	—	—	—	—	—
J. D.	59	M	810	410	480	430	550	590	95	70	50	58	67	67	.068	.099	.060	.078	.070	.065	4.7	3.9	9.1	11.1	9.4	7.0
E. F.	61	M	950	850	870	820	930	950	95	83	84	75	75	65	.056	.054	.054	.051	.045	.038	5.8	5.5	6.4	5.5	6.7	8.6
J. M.	41	M	980	830	770	820	710	590	85	75	72	64	60	55	.056	.058	.060	.051	.055	.061	4.5	7.8	7.2	7.8	7.0	8.7
C. M.	33	M	960	—	840	720	710	810	105	95	94	95	92	93	.065	—	.066	.077	.077	.068	4.4	4.8	5.0	5.0	5.0	6.0
M. N.	69	F	660	490	480	440	—	—	120	105	102	87	80	75	.120	.132	.139	.130	—	—	5.9	5.1	9.9	8.0	—	6.8
A. T.	52	F	870	—	610	380	—	—	105	65	53	50	60	70	.073	—	.053	.080	—	—	4.6	—	9.5	10.2	8.7	8.2
E. W.	62	F	760	460	490	430	340	460	148	92	80	69	69	72	.119	.124	.101	.099	.125	.097	5.2	9.4	7.6	9.4	11.8	12.5
M. W.	51	F	1930	2170	1240	990	1030	790	150	127	110	100	95	90	.059	.044	.066	.076	.069	.086	4.6	4.7	5.2	5.1	4.9	5.0
A. Y.	71	M	760	540	440	460	—	570	127	68	75	75	76	85	.095	.072	.097	.093	—	.086	5.8	8.2	7.8	7.4	—	9.0
P. T.	52	M	640	—	370	450	420	470	110	78	70	70	70	72	.110	—	.119	.098	.106	.097	9.4	10.1	10.6	10.7	10.9	12.1
T. S.	41	M	810	—	510	450	360	—	99	74	68	69	70	—	.072	—	.079	.091	.113	—	5.7	—	9.4	8.9	10.4	—
Mean:			930 ±80*	820 ±170	690† ±68	620† ±59	690† ±70	680† ±55	110 ±5.2	86† ±4.2	79† ±4.4	75† ±4.0	75† ±3.7	76† ±2.1	.078 ±.007	.083 ±.013	.078 ±.007	.082 ±.006	.076 ±.008	.072 ±.005	5.5 ±.34	6.3 ±.59	7.7† ±.52	7.8† ±.60	7.9† ±.72	8.0† ±.65
T. A.‡	49	F	1510	1270	1060	1720	2110	2040	107	90	73	70	73	83	.045	.045	.043	.026	.022	.026	4.2	3.1	5.3	3.8	3.5	3.2
H. H.‡	69	M	1010	—	950	1210	1230	1220	92	75	79	73	70	70	.044	—	.041	.029	.028	.028	5.6	6.5	4.1	3.5	3.2	5.1

* In this and Table III ± values are standard errors of the mean.
† Differs significantly ($p < .05$) from control value.
‡ Subjects T. A. and H. H. reacted in an anomalous manner (see text).

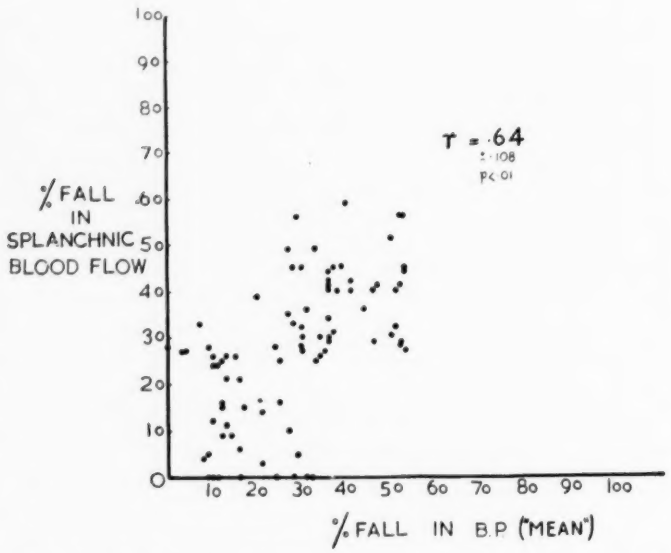

FIG. 2. RELATION BETWEEN FALL IN SPLANCHNIC BLOOD FLOW (E.S.B.F.) AND "MEAN" B.P. AFTER HEXAMETHONIUM

Splanchnic vascular resistance initially was highest in those with the highest systemic blood pressure, confirming the observations of Culbertson, Wilkins, Ingelfinger, and Bradley (19). There was no change following hexamethonium in either normotensive or hypertensive subjects.

Arterial-hepatic venous oxygen difference increased significantly (Figure 1). This increase was entirely due to a drop in hepatic venous oxygen content, for arterial oxygen unsaturation did not change during the course of the study. It coincided in time with the fall in "mean" blood pressure and in E.S.B.F.

"Portal Vein Pressure" (Table II)

In six subjects the "portal venous pressure" measured by the "occluded" hepatic vein technique fell after hexamethonium. The degree of fall in pressure varied considerably and was unrelated to the fall in E.S.B.F.

Observations during Second Hour

In six subjects in whom observations were continued for a second hour, the fall in blood pressure and E.S.B.F. and the increase in arterial-hepatic venous oxygen difference were maintained, with only a slight return towards control values.

Anomalous Results

Two subjects reacted in a different manner (T. A., H. H., Table I). After a brief initial drop, E.S.B.F. rose moderately and was accompanied by a fall in arterial-hepatic venous oxygen difference. Since the blood pressure fell, there was a drop in estimated splanchnic resistance, indicating splanchnic vasodilatation. There was no obvious explanation for this anomalous behaviour.

Changes in Splanchnic Metabolism (Table III)

Splanchnic oxygen consumption calculated by multiplying E.S.B.F. by the arterial-hepatic venous oxygen difference did not change significantly during the hour following hexamethonium.

TABLE II

"Occluded" hepatic vein pressure before and after hexamethonium

Subject	Occluded hepatic vein pressure (mm. Hg)		Fall in E.S.B.F. (% of control value)
	Before C6	After C6	
T. S.	10.2	5.0	55
A. Y.	5.3	4.1	25
E. W.	13.0	10.0	40
P. T.	9.0	5.5	27
C. M.	14.5	2.0	16
C. W.	13.3	1.6	—

Hepatic glucose output. The basal glucose output of 120 mg. per min. per sq. meter agrees well with previous findings (20). There were considerable fluctuations in output following hexamethonium, the mean values being higher than the basal output except at 30 minutes. The changes, however, were not statistically significant.

Arterial blood glucose concentration increased slightly, but again the change was not statistically significant.

Arterial lactic acid concentration rose from 8.2 mg. per cent to a maximum of 9.7 mg. per cent. This change was of doubtful significance.

DISCUSSION

The fall in E.S.B.F. after hexamethonium correlated well with the fall in "mean" blood pressure (Figure 2), and there was therefore little change in calculated splanchnic vascular resistance. Appreciable splanchnic vasodilatation apparently did not occur and splanchnic blood flow fell as a direct consequence of the drop in arterial pressure. This is rather surprising inasmuch as changes in capacity of the splanchnic bed have long been thought to play an important part in the production of the hypotensive state. The present results could have two interpretations. The dose of hexamethonium employed might have failed to block the splanchnic sympathetic ganglia. This could hardly be true of sympathetic ganglia elsewhere, for the general blood pressure fell markedly. Alternatively, splanchnic vasodilatation produced by hexamethonium might have been balanced by splanchnic vasoconstriction, in response to hypotension. For example, the hypotension initiated by changing from the recumbent to the erect posture is prevented by reflex splanchnic vasoconstriction (19). The hypotension following hexamethonium might also be balanced in this way, although this could hardly have been mediated through autonomic ganglia which are presumably already blocked.

Whatever the mechanism, the fall in E.S.B.F. was not associated with overall changes in the capacity of the splanchnic vascular bed.

Changes in splanchnic blood flow could, of course, be due to alterations in resistance in any

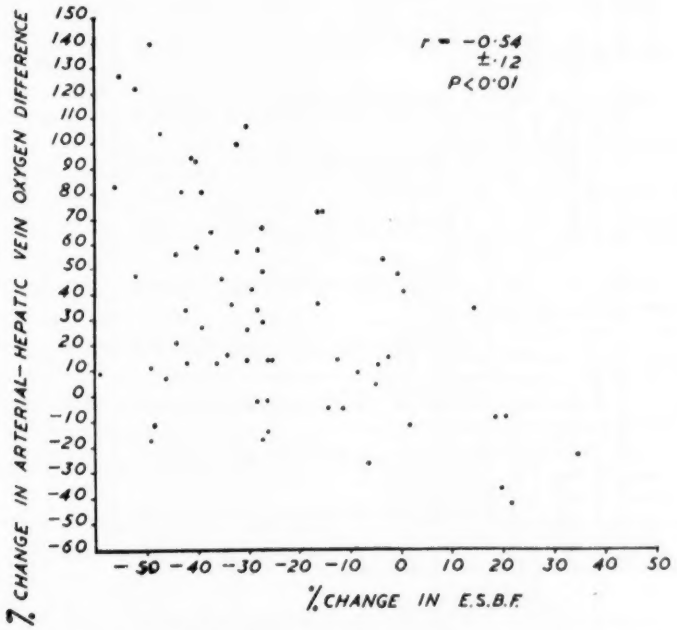

FIG. 3. RELATION BETWEEN CHANGES IN E.S.B.F. AND ARTERIAL-HEPATIC VENOUS OXYGEN DIFFERENCE AFTER HEXAMETHONIUM

TABLE III

*Effect of hexamethonium on splanchnic oxygen consumption, arterial glucose and lactic acid concentrations and hepatic glucose output**

Subject	Age	Sex	Splanchnic O_2 Consumption (*vol./min./m²*)						Arterial Glucose Concentration (*mg./100 ml.*)						Hepatic Glucose Output (*mg./min./m²*)						Arterial Lactic Acid Concentration (*mg./100 ml.*)					
			min. after C6						min. after C6						min. after C6						min. after C6					
			0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60
F. A.	35	M	56	50	58	79	85	82	78	75	90	89	88	94	113	240	211	228	281	234	9.0	6.5	7.5	9.0	6.5	4.5
F. C.	42	M	52	53	37	32	—	38	72	74	75	70	69	69	133	171	104	73	—	98	5.0	6.0	5.5	6.0	6.5	5.0
L. C.	46	F	37	25	36	27	40	37	77	84	85	103	99	100	122	52	284	126	142	78	7.0	7.5	6.0	7.5	10.0	8.5
E. C.	34	M	—	—	—	—	—	—	91	—	94	86	88	77	64	—	—	0	39	0	13.0	7.5	9.0	12.5	9.0	8.5
J. D.	59	M	38	16	44	48	52	41	93	86	81	101	93	84	78	53	86	69	154	147	12.0	11.0	11.0	21.0	16.0	16.0
E. F.	61	M	56	47	56	45	62	83	77	—	76	68	81	107	275	—	235	82	223	190	6.5	7.0	8.0	6.0	—	5.5
J. M.	41	M	44	65	55	65	49	—	82	79	93	93	81	84	235	125	185	82	177	59	8.0	6.5	5.0	8.0	9.0	6.0
C. M.	33	M	42	—	42	36	35	48	86	86	85	88	80	77	96	—	109	72	57	170	5.0	6.0	7.0	7.0	7.0	5.0
M. N.	69	F	39	—	48	36	—	—	95	92	94	82	92	95	158	118	125	123	—	—	12.0	12.0	9.0	10.0	10.0	12.5
A. T.	52	F	40	—	58	39	—	—	79	83	—	87	98	104	87	—	—	46	—	—	4.3	8.5	6.5	6.0	6.0	5.0
E. W.	62	F	40	43	37	40	40	60	86	85	89	81	86	95	46	60	64	65	126	184	10.5	13.5	17.5	19.5	25.0	25.5
M. W.	51	F	88	102	65	51	51	39	93	88	98	96	95	94	77	217	149	79	124	55	5.5	7.0	7.5	9.5	11.5	12.0
A. Y.	71	M	44	45	34	34	—	42	—	—	—	—	—	—	—	—	—	—	—	—	5.1	6.5	8.5	5.0	4.0	4.8
P. T.	52	M	60	—	40	48	46	57	81	87	86	87	81	86	96	—	44	45	64	52	5.5	4.5	5.7	6.5	5.0	8.0
T. S.	41	M	46	—	48	40	37	—	78	—	78	86	80	—	65	—	71	58	32	—	8.0	—	6.0	9.0	9.5	—
T. A.	49	F	64	40	56	65	74	65	91	92	93	93	95	93	121	102	74	206	211	163	7.5	7.5	7.5	10.0	10.0	8.0
H. H.	69	M	56	—	39	42	39	62	91	87	87	87	81	87	151	—	152	133	221	183	16.0	20.0	13.6	8.0	9.5	10.0
Mean			50 ±3.4	49 ±7.4	47 ±2.5	45 ±3.8	51 ±4.6	54 ±4.8	84 ±1.7	84 ±1.6	87 ±1.9	87 ±2.3	87 ±2.0	90 ±2.8	120 ±15.4	126 ±23.3	135 ±19.7	97 ±13.4	143 ±21.9	124 ±19.7	8.2 ±0.82	8.6 ±0.99	8.2 ±0.76	9.4 ±1.09	9.7 ±1.24	9.0 ±1.37

* None of the means differs significantly ($p < .05$) from control value.

of three systems, namely hepatic arterioles, portal vein radicles or intestinal arterioles. All parts of the splanchnic vasculature need not react similarly in producing the overall unchanged splanchnic vascular resistance after hexamethonium.

Theoretically, constriction in the portal vein radicles and dilatation in the intestinal arterioles might occur without any change in overall vascular resistance. Portal venous pressure would then be expected to rise. Hexamethonium, however, lowered "portal venous pressure," suggesting that there was no significant portal venous constriction.

On the other hand, dilatation in the portal vein radicles with intestinal arteriolar constriction might occur, and again overall vascular resistance would be unaltered. In this case there would be a fall in portal venous pressure, as in fact occurred. However, it seems unlikely that hexamethonium would cause arteriolar vasoconstriction.

Hepatic arteriolar dilatation, by raising intrasinusoidal pressure, might decrease portal venous inflow (21) and hence total splanchnic blood flow. But in this case portal venous pressure would be expected to rise.

Finally, a fall in splanchnic blood flow without any change in vessel caliber would lead to a fall in portal venous pressure, with unchanged splanchnic vascular resistance. This seems the most likely explanation of the present results.

Additional evidence for the fall in splanchnic blood flow was afforded by the arterial-hepatic venous oxygen difference. If oxygen utilization by the splanchnic area is unchanged (Table II), then the arterial-hepatic venous oxygen difference should vary inversely with the splanchnic blood flow. This in fact proved to be the case (Figure 3). The mean rise in arterial-hepatic venous oxygen difference was 31 per cent, a similar figure to that obtained for E.S.B.F. by the B.S.P. clearance technique.

The effects of hexamethonium on the splanchnic area appear to be limited to changes in circulation. Changes in hepatic glucose output and arterial glucose concentration did not occur. The failure of arterial lactic acid concentrations to rise appreciably indicates that the basal state of the subjects was maintained, since blood lactic acid increases rapidly following muscular activity or release of epinephrine (22).

SUMMARY

The effect of hexamethonium bromide (1 mg. per Kg. intramuscularly) on the splanchnic circulation, splanchnic oxygen consumption, hepatic glucose output and arterial lactic acid concentration was measured in 17 subjects.

In 15 subjects splanchnic blood flow fell in proportion to the fall in arterial blood pressure. No change occurred in splanchnic vascular resistance, indicating that there was no splanchnic vasodilatation.

In two subjects splanchnic blood flow rose, together with a fall in splanchnic vascular resistance.

Splanchnic oxygen consumption, hepatic glucose output and arterial glucose and lactic acid concentrations were not significantly altered.

ACKNOWLEDGMENTS

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THE CHOLESTEROL-ESTERIFYING ENZYME OF HUMAN SERUM. I. IN LIVER DISEASE [1]

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(Submitted for publication November 6, 1952; accepted May 1, 1953)

The presence of an enzyme in normal human blood serum capable of esterifying cholesterol *in vitro* was first reported by Sperry (1). He found that when serum was incubated its free cholesterol decreased while the total cholesterol was unchanged. It was concluded that esterification of some of the free cholesterol had taken place through the action of an enzyme, as the effect was abolished by heating the serum to 55 to 60° C. prior to incubation. Hemolysis also inhibited the reaction as did bile salts.

It seemed probable that the liver might be connected in some way with the production of this enzyme, as the ratio of free to ester cholesterol in the serum is often abnormal in hepatic disorders. Therefore, in some types of liver disease decreased enzyme activity might be expected. This was indeed found to be the case and a preliminary report on a small series of patients was made in 1949 (2). Similar results were published simultaneously from the University of Madrid (3).

The behavior of this enzyme has been studied in 254 patients with liver disease and an attempt has been made to determine wherein this test may supplement other tests of liver function.

METHODS

Blood was obtained from 20 normal subjects serving as controls and from 254 patients with liver disease divided as follows: Acute hepatitis—76; chronic hepatitis—19; cirrhosis—69; biliary tract disease—45; carcinoma of pancreas or biliary tract—21; Hodgkin's disease and metastatic carcinoma of the liver—17; miscellaneous liver disease—7. As a rule multiple samples were secured from each individual to determine enzyme activity throughout the course of the disease. Blood was usually drawn from the fasting subject. Samples that were visibly hemolyzed were discarded because of the inhibiting effect of hemolysis on enzyme activity.

[1] This investigation was aided by research grant H39 from the National Heart Institute, U. S. Public Health Service.

The amount of total and free cholesterol in a sample of serum was determined by the method of Schoenheimer and Sperry (4) before and after incubation of the serum at 37° C. for 24 hours. The difference between the values for free and total cholesterol was assumed to represent cholesterol ester. With incubation the amount of free cholesterol usually fell. The percentage decrease was calculated. As a matter of convenience this drop has been expressed by *enzyme units*. One unit is defined as the amount of enzyme activity capable of producing a one per cent decrease in the original level of free cholesterol.

Other tests of liver function used for comparison were the serum bilirubin, alkaline phosphatase, and cephalin flocculation. These were performed in the laboratory for routine chemistry by standard methods.

The following were accepted as normal values for the serum: total cholesterol, 150–300 mg. per cent; ester cholesterol, 70 per cent or more of the total; activity of the cholesterol-esterifying enzyme, 30 units or more; bilirubin, less than 1.0 mg. per cent; alkaline phosphatase, less than 6.0 Bodansky units. The cephalin flocculation is normally negative.

Normal Subjects

In 20 normal subjects the total serum cholesterol ranged from 156 to 268 mg. per cent, of which 71 to 75 per cent was esterified. The free cholesterol before incubation ranged from 40 to 75 mg. per cent. With incubation, enzyme action caused a drop of 31 to 50 per cent (31 to 50 units) in the free cholesterol. In 17 of of the 20 subjects the decrease was 33 to 44 units.

Hepatitis

There were 76 patients with viral hepatitis. About 25 to 30 per cent of these were probably homologous serum hepatitis. From the standpoint of enzyme activity there was no apparent difference between patients with infectious hepatitis and those considered to have homologous serum hepatitis.

Enzyme activity varied according to the week of the disease as shown in Figure 1. Admitting the obvious difficulty in determining the duration of the disease with its insidious onset, enzyme activity was always low in the first week of hepatitis. During the second week 32 of 39 blood samples also had low enzyme activity. Thereafter enzyme activity returned to normal so that in the fourth week, normal values were obtained in 21

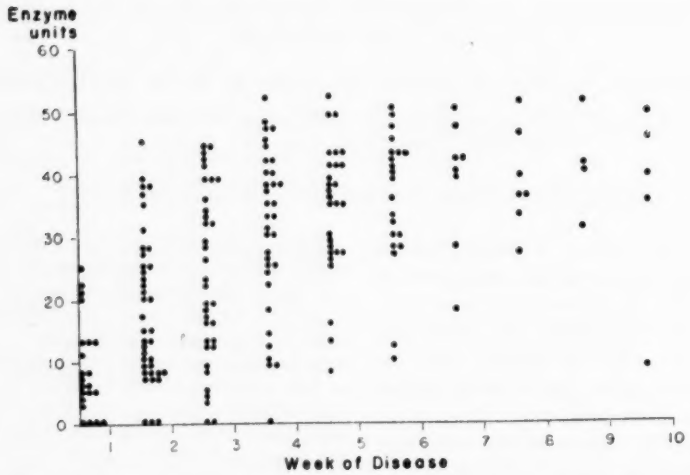

FIG. 1. ENZYME ACTIVITY IN VIRAL HEPATITIS ACCORDING TO THE DURATION OF THE DISEASE

Each dot represents one determination.

of 34 samples and in the sixth week in 16 of 21 samples. An increase in enzyme activity of 25 or 30 units in a week or two was not uncommon. The greatest increase encountered was a rise from 7 enzyme units to 42 units in one week.

The return of enzyme activity to normal occasionally preceded other evidence of improvement. The finding of normal enzyme activity early in the disease was a good prognostic sign. Increasing enzyme activity was associated with a good prognosis; falling enzyme activity with a poor prognosis. Only one patient who showed a significant fall in enzyme activity recovered. This is included in Figure 2 which shows typical examples of fluctuations in enzyme activity.

There were eight deaths in this group of 76 patients. All of the fatalities occurred in patients who had less than 10 enzyme units in the serum. On the other hand 23 patients with less than 10 enzyme units recovered.

In hepatitis when a normal percentage of esterified cholesterol was found, normal enzyme activity was encountered except in one case with 27 enzyme units. However, normal enzyme activity was noted in 15 patients in whom the proportion of esterified cholesterol was subnormal (below 70 per cent). Enzyme activity which

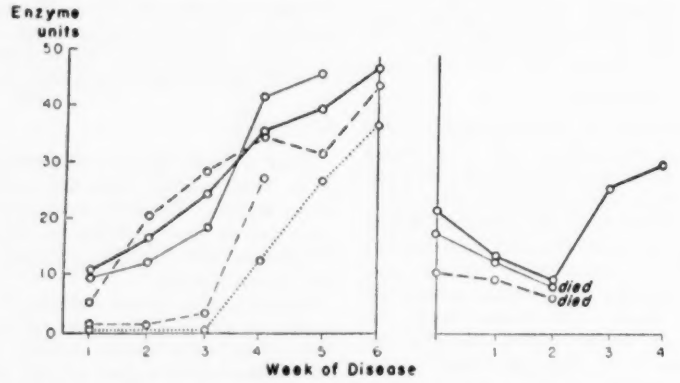

FIG. 2. EXAMPLES OF REPEATED DETERMINATIONS OF ENZYME ACTIVITY IN SELECTED PATIENTS WITH VIRAL HEPATITIS

A rise in enzyme units was associated with a good prognosis; a significant fall with a poor prognosis, with the exception illustrated.

TABLE I

Chemical data on hepatitis patients with negative or slightly positive cephalin flocculation tests

Case	Sex	Age	Serum bilirubin	Cephalin flocculation	Alkaline phosphatase	Total cholesterol	Cholesterol ester	Enzyme units
			mg. %		*B.U.*	*mg. %*	*%*	
1	F	41	4.3	±	24.9	615	15	0
2	F	18	3.1	0	10.1	319	72	27
3	F	35	21.0	0	3.8	268	30	10
4	M	28	6.5	0	14.7	285	27	17
5	M	28	3.6	0	6.7	238	30	25
6	M	43	11.8	0	7.7	129	39	9
7	M	18	11.2	0	1.1	254	28	12
8	F	18	18.2	+	7.3	222	30	19
9	M	50	3.2	0	10.7	234	30	13
10	M	51	16.4	0	8.4	250	40	22
11	M	27	16.5	0	7.1	279	26	11
12	M	39	17.1	0	11.1	252	50	19

was rising might become normal 4 to 14 days before a normal serum cholesterol partition was found.

In 64 of the 76 patients with hepatitis the determination of enzyme activity added nothing diagnostically as the disease was clearly evident. All of these 64 patients had a positive cephalin flocculation and an elevated serum bilirubin. In a number of cases there was an increase in serum alkaline phosphatase or serum globulin. However, in the remaining 12 cases, the determination of enzyme activity proved diagnostically helpful. In these the cephalin flocculation test was negative in 10 and faintly positive in 2. Significant data on these 12 patients are given in Table I.

In Case 1 surgical exploration was planned but deferred because of the absence of enzyme activity. Within six weeks the patient felt well and the serum bilirubin had fallen nearly to normal as had the phosphatase. The serum cholesterol had dropped to 441 mg. per cent, the enzyme units had risen to 21, and the cephalin flocculation had become 3+. Recovery thereafter was uneventful. Case 2 was improving when first seen and the enzyme units were nearly normal. This was also true of Case 5. Case 3 was treated conservatively and improved promptly. On Case 4 a laparotomy was performed for possible biliary tract obstruction. None was found. Autopsy revealed a viral hepatitis. Case 6 was also operated upon and died shortly thereafter. Biopsy and autopsy showed a virus hepatitis. Cases 7 and 8, both homologous serum hepatitis, improved steadily. Cases 9 to 12 were diagnosed pericholangitic hepatitis on biopsy.

Chronic Hepatitis

In 19 patients with this diagnosis 11 had less than 30 units of enzyme, and 8 had normal enzyme activity. The decrease in enzyme activity was less marked than in acute hepatitis. Four patients had less than 20 enzyme units, and in 7 the values ranged from 21 to 29 units.

The serum bilirubin, alkaline phosphatase, and globulin were usually increased. Three of the group had negative cephalin flocculation tests. The enzyme activity was normal in two of these but in one it was only 13 units. This patient ultimately died of hepatic insufficiency. The percentage of cholesterol esters was less than 70 per cent in 18 patients, of whom 11 had decreased enzyme activity and 7 had normal amounts of enzyme. The single patient with a normal proportion of esterified cholesterol had 40 units of enzyme.

In chronic hepatitis, as in acute hepatitis, enzyme activity of less than 10 units seemed of prognostic significance. There were two deaths. Shortly prior to death, one of these patients had 9 enzyme units and the other only 1 unit. One patient who had 9 enzyme units on admission gradually improved.

Cirrhosis

Low enzyme activity was found in 42 and normal activity in 27 of 69 patients with Laennec's cirrhosis. In general, the more severely damaged the liver, as shown by other liver function tests, the more likely was decreased enzyme activity to be found (Table II). While there was a general correlation between enzyme activity and the percentage of esterified cholesterol in the serum, it was not exact. In eight patients with a normal ratio of

TABLE II

Laennec's cirrhosis

		Enzyme activity	
Number of cases:		Low 42	Normal 27
Total cholesterol	—low	18	7
	normal	18	19
	high	6	1
Per cent esterified cholesterol	—low	34	11
	normal	8	16
Serum bilirubin	—increased	32	14
	normal	9	9
Cephalin flocculation	—positive	29	14
	negative	11	12
Serum alkaline phosphatase	—increased	8	8
	normal	31	16
Albumin/globulin ratio	—abnormal	20	13
	normal	10	10

TABLE III

Biliary tract disease

Diagnosis	No. of patients	Enzyme activity		Total cholesterol Low	Total cholesterol High	Ester cholesterol decreased	Cephalin flocculation positive	Bilirubin increased	Alkaline phosphatase increased
Cholecystitis and cholangitis	19	normal	14	2	0	1	1	11	11
		low	5	1	1	3	3	3	5
Obstruction due to stone	8	normal	6	0	1	5	0	6	6
		low	2	0	2	2	0	2	2
Biliary cirrhosis	10	normal	2	0	1	0	0	2	2
		low	8	0	6	6	7	8	8
Common duct stricture	6	normal	5	0	1	5	0	4	5
		low	1	0	1	1	1	1	1
Congenital atresia	2	normal	1	0	0	1	0	1	1
		low	1	0	1	1	0	1	1
Total	45	normal	28	3	14	25	12	39	42
		low	17						

cholesterol ester, low enzyme activity was detected, while in 11 patients with a decreased percentage of ester, normal enzyme values were obtained.

In this disease there was very little correlation between the cephalin flocculation and enzyme activity. The flocculation test was positive in 43 patients, 29 of whom had decreased and 14 normal amounts of enzyme. In the 23 cases with negative cephalin flocculation, 12 had normal and 11 decreased enzyme activity.

Enzyme activity appears not to have diagnostic value in cirrhosis. From the standpoint of prognosis, however, determination of enzyme activity may be of value. There were 11 deaths in this series. In two instances enzyme activity was normal. Both of these patients died from ruptured esophageal varices. In nine of the fatal cases enzyme activity was low. Death was due to hepatic insufficiency in seven of these, to hemorrhage in one, and to primary liver carcinoma in the remaining case.

Biliary Tract Disease

The 45 patients in this group were subdivided as follows: Cholecystitis and cholangitis—19 cases; obstructive jaundice due to common duct stone—8 cases; biliary cirrhosis—10 cases; cicatricial stricture of the common duct—6 cases; congenital atresia of bile ducts—2 cases (Table III). It was realized that this separation at times was arbitrary and that overlap might occur.

Decreased enzyme activity was noted in 17. This reduction was slight (26 to 28 enzyme units) in cholecystitis. In general, the lowered enzyme activity paralleled a positive cephalin flocculation test and probably was the result of biliary cirrhosis.

High levels of total serum cholesterol were found in biliary cirrhosis and with obstruction due to an increase in free cholesterol and a corresponding decrease in esterified cholesterol. There was poor correlation between decreased enzyme activity and a low cholesterol ester fraction.

The serum alkaline phosphatase was high in 42 and the serum bilirubin in 39.

Obstructive Jaundice Due to Neoplasm

There were 21 patients with jaundice resulting from an obstructing neoplasm; pancreatic carcinoma in 14, carcinoma of bile duct in 2, and of gall bladder in 2. The remaining 3 cases were carcinoma of stomach, lymphosarcoma, and a benign papilloma of the common bile duct.

The serum bilirubin and alkaline phosphatase were increased in all. The cephalin flocculation test was negative in 18, positive in 1, and not done in 2. Total serum cholesterol values were high in 15. The percentage of esterified cholesterol was subnormal in 19. Enzyme activity was decreased in 16.

Hodgkin's Disease and Metastatic Involvement of Liver

Of the 17 patients in this group, enzyme activity was decreased in 7. The total serum cholesterol was low in 6, high in 6. Esterified cholesterol was decreased in 13.

Two patients had only cholesterol determinations and no other liver function tests. In the remaining 15 cases, the cephalin flocculation test was positive in 6, the serum bilirubin elevated in 6, and the alkaline phosphatase was high in 13.

Miscellaneous Liver Disease

There were seven patients in this group. Three of these had liver abscess. There was one case of each of the following: infectious mononucleosis, miliary tuberculosis, carbon tetrachloride poisoning, cavernomatous transformation of the portal vein. These cases are obviously too few for detailed analysis. Enzyme activity was normal in all.

DISCUSSION

It may fairly be asked whether determination of the activity of the cholesterol esterifying enzyme yields diagnostic information beyond that af-

forded by the usual serum cholesterol partition. We believe that the answer is in the affirmative in a number of situations.

There are two ways in which a decreased proportion of esterified cholesterol in the serum may arise: 1) an actual decrease in the amount of cholesterol ester without a corresponding decrease in free cholesterol, or 2) an actual increase in the amount of free cholesterol without a corresponding increase in ester cholesterol. Determination of enzyme activity helps to differentiate between these two causes of a low percentage of esterified cholesterol. This is particularly true in obstructive jaundice due to gall-stones where the percentage of ester cholesterol is usually low as a result of an accumulation of free cholesterol in the blood. In these cases the enzyme activity is usually normal. Less often this is seen with an obstructing carcinoma, chronic hepatitis or biliary tract disease. In cirrhosis the lack of correlation between enzyme activity and the ratio of cholesterol ester should be emphasized. Furthermore, in the course of acute hepatitis the return to normal of enzyme values before the restitution of a normal cholesterol partition is of interest.

The value of the determination of enzyme activity in the prognosis of hepatitis and cirrhosis has been stressed. All the deaths due to acute or chronic hepatitis in this series have occurred in patients with less than 10 enzyme units in the serum. All deaths due to hepatic insufficiency in the patients with Laennec's cirrhosis have also occurred in the group with low enzyme activity.

The diagnostic applications of the test may be summarized as follows: Decreased enzyme activity is found uniformly in the early stages of acute hepatitis. In most instances the diagnosis is apparent without the aid of the determination of enzyme activity, particularly as a positive cephalin flocculation test points strongly to damage of the liver parenchyma. However, in 12 of the 76 cases of hepatitis the cephalin flocculation test was negative and the finding of low enzyme values in these cases threw the weight of diagnostic evidence toward hepatitis. Included in this category were the four cases of pericholangitic hepatitis.

In chronic hepatitis low levels of enzyme activity are often found but this does not help to differentiate the condition from cirrhosis, either portal or biliary. In Laennec's cirrhosis, the serum of about 60 per cent of the patients shows decreased enzyme activity but this is not a diagnostic aid as a rule. In biliary cirrhosis 8 of the 10 patients were found to have decreased activity of the enzyme.

In contrast to hepatitis and cirrhosis enzyme activity was usually normal in cholecystitis, cholangitis, obstructive jaundice due to gall-stones, and cicatricial deformity of the common ducts. However, in obstructive jaundice due to neoplasm decreased enzyme activity was noted in 16 of the 21 cases.

It had been hoped originally that the determination of enzyme activity would serve to differentiate between parenchymal liver disease and extrahepatic obstruction. While this proved to be true in disease of the biliary tree other than neoplasm, its failure in obstructive jaundice due to tumor proved disappointing. Some type of inhibiting substance in the serum has been postulated. It seemed possible that this might be cholic acid as Sperry (1) had shown that bile salts would inhibit the activity of the enzyme. So far, we have not been able to demonstrate that this is the mechanism involved in these cases.

In analyzing further the patients with obstructive jaundice due to neoplasm whose serum showed decreased enzyme activity, an interesting tetrad of chemical findings emerged. In 12 of the 16 patients the following combination was found: 1) serum bilirubin 10 mg. per cent or more; 2) serum alkaline phosphatase 10 Bodansky units or more; 3) total serum cholesterol 300 mg. per cent or more; 4) negative cephalin flocculation. In the remaining 238 cases in the total series, this tetrad was encountered in only six. Four of the six patients had obstructive jaundice due respectively to neoplasm, cicatrix, stone, and congenital atresia of the bile ducts. (The patient with the neoplasm had normal enzyme activity.) One patient was diagnostically puzzling but finally classified as having biliary cirrhosis. In the sixth patient who had viral hepatitis, the tetrad was found transiently a week or two before death. Thus in the 18 cases with this tetrad, a surgical condition was present in 16.

SUMMARY

1. Observations on the activity of the cholesterol-esterifying enzyme have been made in 254 cases of liver disease.

2. In hepatitis, enzyme activity was uniformly low early in the course of the disease. Determination of enzyme activity was of diagnostic value in the 16 per cent of the cases in this group who had negative cephalin flocculation tests. Prognostically, decreasing enzyme activity was an ominous sign. All the deaths in this group occured in patients with less than ten enzyme units.

3. Diminished enzyme activity was found in 42 of 69 cases of Laennec's cirrhosis. The test had little diagnostic value in this condition but from a prognostic standpoint it was noted that deaths from hepatic insufficiency all occurred in the group with low enzyme activity.

4. In biliary cirrhosis, enzyme activity was usually low.

5. Normal enzyme activity was usually found in patients with cholecystitis, cholelithiasis and common duct stricture. The test was often of diagnostic value in these conditions.

6. In 16 of 21 cases of obstructive jaundice due to neoplasm enzyme activity was decreased. In 12 of the 16 there was encountered a tetrad consisting of: serum bilirubin above 10 mg. per cent, alkaline phosphatase above 10 Bodansky units, total cholesterol above 300 mg. per cent, and a negative cephalin flocculation test. This combination was found in only six other patients and four of these had obstructive jaundice. Thus, of the 18 patients showing the tetrad, extrahepatic obstructive jaundice was present in 16.

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FACTORS INFLUENCING THE DIURETIC RESPONSE OF SEATED SUBJECTS TO THE INGESTION OF ISOTONIC SALINE SOLUTION

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The ingestion of isotonic saline solution, long considered to have little or no immediate effect on urine flow (1), has recently been shown by Blomhert (2) to be followed by brisk water diuresis. Further, several investigators have demonstrated that similar diuresis may result from intravenous infusion of isotonic saline solution (2, 3, 4). Examination of the latter experiments reveals at least three factors which appear to be of importance in determining whether water diuresis ensues: hydration, posture and time of day. Thus Ladd (3) found that diuresis following saline infusion occurred only if the subjects had been prehydrated eight to thirteen hours before the infusion was given. A previous study from this laboratory (4) showed that diuresis occurred in recumbent subjects but not in those quietly seated in a chair. Blomhert (2) did not observe diuresis when infusion was accomplished at night, although it occurred regularly under the same conditions during the day.

Since Blomhert's studies were made only on recumbent subjects and no mention of posture is to be found in other reports concerning the response to ingested saline, it was deemed worthwhile to ascertain whether diuresis might be induced, and if so, under what circumstances, following saline ingestion by seated subjects.

METHODS

The subjects were essentially healthy male adults free of cardiovascular, renal and endocrine disease. Except for standing to void at appropriate intervals, they remained quietly seated in a straight backed chair during the study. Methods of analysis have been described in recent publications from this laboratory (4, 5).

RESULTS

A. Observations on the response to saline ingestion in trained subjects with and without prior hydration

The subjects were three physicians accustomed to the procedures involved and capable of voluntarily emptying their bladders at short intervals. In the first study the subjects drank approximately one liter of fluid with breakfast and collected urine at intervals until the flow had declined from its peak rate to less than 2 ml. per min. At this time one liter of 0.9 per cent saline was ingested within 10 minutes. In each instance the rate of urine flow increased briefly, reaching peak flows of 3.3, 4.7 and 5.9 ml. per min. in the third quarter hour and returning to the initial rate at the end of an hour. Data for one subject are shown in Figure 1. The other two subjects showed similar changes. Urinary electrolyte and creatinine concentrations as well as specific gravity fell. Urea and ammonia excretion were measured in one subject. The total solute concentration, estimated as the sum of urea plus twice the sodium, potassium, and ammonia (in order to include their accompanying anions), at the time of maximum flow was 185 mM per L., definitely hypotonic to extracellular fluid. Creatinine excretion remained relatively constant [1] suggesting that the diuresis resulted from diminished tubular reabsorption of water rather than increased filtration.

[1] Since venipuncture, no matter how skillfully performed, might constitute an antidiuretic stimulus, no blood was obtained for analysis. However it is improbable that significant variation in serum creatinine occurred during the short time intervals involved. The validity of creatinine clearance as an index of glomerular filtration rate in normal man has been confirmed (6).

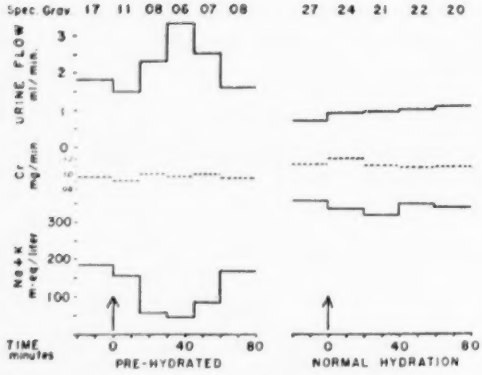

FIG. 1. THE EFFECT OF THE INGESTION OF ONE LITER OF 0.9 PER CENT SODIUM CHLORIDE SOLUTION ON URINARY FLOW, CREATININE EXCRETION, SPECIFIC GRAVITY AND ELECTROLYTE CONCENTRATION WITH AND WITHOUT PRE-HYDRATION

Saline was ingested in each case at the time indicated by the arrow. Only the last two digits of the specific gravity are shown.

When the saline was ingested under identical conditions save that fluids at breakfast were restricted to 250 ml. there occurred only a slight augmentation in urine flow (Figure 1). The other subjects reacted similarly.

At the end of one hour, when it was apparent that a diuretic response was not forthcoming, a second liter of saline was ingested during a period of ten minutes. Urine collections were continued for two hours. Diuresis did not occur. Endeavors to administer larger volumes of saline were frustrated by the appearance of mild cramps and frank diarrhea.

These observations indicate that in seated subjects who are on the descending limb of a previous water diuresis, transient but definite water diuresis may follow the ingestion of one liter of isotonic saline solution, but that in normally hydrated, seated subjects neither one nor two liters of ingested saline leads to such a response.

B. Observations on normally hydrated subjects with and without prior salt loading

Without previous preparation each of four subjects came to the laboratory one to two hours after a breakfast in which fluids were restricted to 250 ml. Following the collection of timed control urine specimens, blood was obtained for analysis and the subject drank one liter of 0.9 per cent saline solution within 10 to 20 minutes. Urine was obtained at approximately hourly intervals thereafter for the next three hours. During the remainder of the afternoon, but before 6 P.M., the subject drank sufficient 0.9 per cent saline solution, to constitute together with the initial liter, a load of 6.2 mEq. per Kg.[2] The subjects ate their usual meals (with salt as desired) and took whatever additional liquids they wished. The following morning the procedure of the preceding day insofar as venipuncture, urine collections and saline ingestion were concerned was repeated.

In each instance when saline was ingested without antecedent administration of extra salt there was a minor increase in urine flow associated with an *increased* concentration of salt therein (Table I).

Following the salt loading there was in each instance a gain of weight (average 0.9 Kg.) ascribable to an increase in the volume of extracellular fluid (Table II). Although the initial urine flow was distinctly greater than on the preceding day, so was the rate of sodium chloride excretion, as a result of which the urinary electrolyte concentration differed little from that which obtained 24 hours earlier.

Following the ingestion of saline, diuresis commenced promptly, reaching peak flows of 5.0 to 8.9 ml. per min. within an hour and accompanied by a sharp fall in the concentration of electrolytes, in creatinine U/P ratio and in urinary specific gravity. Creatinine clearances exhibited no consistent changes. In one subject in whom urea concentration was determined, the sum of the concentration of urea plus $2(Na + K)$, an estimate of the total urinary solutes, had declined to 152 mM per L., at the time of maximum diuresis, or approximately one-half the osmolarity of extracellular fluid.

In the other three experiments hypotonicity of the urine to the plasma at the time of maximum diuresis may be inferred in view of the fact that the concentration of the major urinary electrolytes ($2 Na + K$) was between 102 and 175 mM per L.

[2] In the case of subject WHB the salt loading was accomplished three days later rather than on the same day.

TABLE I

The effect of the ingestion of isotonic saline solution on urine flow and specific gravity, concentration of electrolytes, creatinine U/P ratio and clearance, and sodium excretion, before and after pre-loading with saline

Subject Period	Time	Urine							
		Flow	Spec. Grav.*	Na	K	Cl	Cr	C_{cr}	Na
	Minutes	*ml./min.*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*U/P ratio*	*ml./min.*	*micro-Eq./min.*
WHB	Pre†	0.5	26	150	94	220	276	138	75
Control	+ 60	0.7	24	180	107	264	267	187	120
	+123	0.7	24	143	166	304	249	174	97
	+189	1.0	22	160	178	312	129	129	155
After	Pre	2.4	15	240	38	265	75	180	584
pre-	+ 33	8.9	04	77	11	80	19	166	680
loading	+ 68	7.0	06	93	15	101	22	154	655
	+100	2.7	14	214	49	222	57	153	588
	+138	2.2	14	202	61	237	68	150	440
CT	Pre	0.3	30	169	110	232	347	104	49
Control	+ 60	0.7	21	202	50	216	174	122	145
	+120	0.6	24	266	82	293	190	114	155
	+180	0.7	24	256	83	326	180	126	184
After	Pre	2.1‡	13	186	24	216	70‡	147‡	398‡
pre-	+ 31	7.7‡	06	69‡	6‡	66‡	19‡	147‡	532‡
loading	+ 67	8.7	04	50	5	51	16	137	438
	+102	2.9	13	187	21	192	51	148	545
	+135	3.0	13	200	25	223	51	153	600
AC	Pre	0.7	22	90	96	141	180	126	60
Control	+ 60	1.0	18	93	92	152	122	122	95
	+120	1.0	19	114	105	190	115	115	118
After	Pre	1.7	15	137	33	156	79	134	228
pre-	+ 30	3.5	08	67	22	72	39	136	233
loading	+ 60	5.0	06	47	16	47	24	119	233
	+ 90	1.6	15	201	44	215	78	125	329
	+120	2.1	14	182	48	210	60	126	383
RD§	Pre	0.4	17	79	107	114	270	108	31
Control	+ 60	0.6	22	53	138	122	197	118	32
	+123	0.9	22	100	89	172	143	129	92
	+163	1.0	22	128	105	205	125	125	121
After	Pre	2.4	11	143	24	147	55	132	341
pre-	+ 30	1.3	20	214	34	211	100	130	286
loading	+ 60	6.6	03	51	8	50	21	138	334
	+ 90	2.3	14	184	32	193	60	138	428
	+120	3.5	11	131	30	146	39	137	459

* Only the last two digits are given.

† Pre = Control period before saline ingestion.

‡ The actual values obtained for these two periods indicate incomplete bladder emptying at the end of the control period and have been corrected by creatinine excretion on the assumption that the actual creatinine clearance remained constant.

§ Urinary urea + ammonia concentration in subject RD was 488, 406, 371 and 351 mM/L. in the control study and 88, 131, 34, 88 and 63 mM/L. in the study after pre-loading with salt.

C. Observations on hydropenic subjects with and without prior salt loading

In order to determine the effect of prior salt loading under conditions of moderate hydropenia each of the five subjects was studied in the "basal" state, *i.e.*, deprived of food and fluid overnight, prior to ingesting the test dose of saline. In none of these subjects did diuresis follow the ingestion of saline prior to pre-loading with salt solution. Following such pre-loading, however, two of the subjects responded to saline ingestion with diureses identical to those of non-hydropenic subjects, achieving peak flows of 6.5 and 8.0 ml. per min. with corresponding declines in specific grav-

ity and electrolyte concentration. The remaining three subjects exhibited little or no change in urine flow after saline ingestion. Thus it would appear that in the presence of an osmotic stimulus to antidiuresis from dehydration, saline ingestion may or may not be followed by an increased flow of dilute urine even when the subject has been pre-loaded with salt.

DISCUSSION

Blomhert has clearly shown that water diuresis may follow the ingestion of isotonic saline solution by normally hydrated recumbent subjects (2). The observations reported here, all made on seated subjects, may be summarized in tabular fashion according to 1) the state of hydration, 2) the prior ingestion of a salt-load, and 3) the occurrence of diuretic response:

State of hydration	Previous salt loading	Diuretic response
Slight hydropenia	None	0/5
Normal	None	0/7
Slightly increased	None	3/3 transient, slight
Slight hydropenia	Yes	2/5 brisk
Normal	Yes	4/4 brisk

The absence of water diuresis following the ingestion of one to two liters of isotonic saline by hydrated seated subjects was not surprising in view of the fact that the intravenous infusion of three liters of isotonic saline is similarly ineffective in seated subjects (4).

The observation that when antidiuretic activity presumably was relatively low (as when trained subjects were on the descending limb of a previous water diuresis), definite if slight and transient water diuresis followed the ingestion of one liter of isotonic saline, although this did not occur when these subjects ingested saline under conditions of normal hydration, suggests an interplay of forces upon the mechanism or mechanisms involved: normal hydration and the sitting position constituting slight antidiuretic stimuli and saline ingestion a moderate diuretic stimulus. These subjects, by virtue of their familiarity with the procedures involved, may be considered to have been free of significant emotional antidiuretic stimuli.

Since there is no reason to believe that water is preferentially absorbed from the gastrointestinal tract when isotonic saline is ingested, it is unlikely that alterations in the effective osmotic pressure of the plasma and extracellular fluid explain the diuresis observed. The suggestion has been made on the basis of the studies cited above (3, 4) as well as others (7, 8) that critical changes in the volume or distribution of the extracellular fluid or alternatively in plasma volume or distribution, may mediate true water diuresis (9).

The inability of normal man rapidly to dispose of a large isotonic saline load affords a simple means of further examining this hypothesis through the administration of such a load on the day preceding the test dose of saline. Although such a load, with the excess of chloride, might conceivably elevate serum chloride levels, no changes of significance were noted (Table II). The decline in serum total proteins, hemoglobin and hematocrit all suggest an expansion of plasma volume. The average gain of 900 Gm. in weight above that of the preceding morning is readily ascribable to an expansion of extracellular fluid volume in both interstitial and plasma compartments. In contrast to sitting subjects receiving isotonic saline intravenously who frequently exhibit slight pitting edema of the legs, these subjects who received saline on the preceding day, awoke on the next morning with perceptible puffiness about the eyes and no edema of the legs. The impression is thus gained that the distribution of the presumed 900 ml. increase in extracellular volume was different from that obtaining when a similar or even larger expansion was accomplished in the sitting position without an intervening period of recumbency.

Whatever the difference may be, however, it is clear that the pre-loaded subjects, being seated in a chair and normally hydrated, developed a prompt water diuresis when they ingested a liter of saline on the following morning. This did not occur in any instance under comparable circumstances when the subjects were not pre-loaded with salt. The fact that three of the five subjects who were given saline under the same conditions save for the fact that they had had no fluids for 12 hours, failed to exhibit diuresis, suggests that the osmotic stimulus to antidiuresis thus present from hydropenia was sufficient to prevent the response. Such an interplay of factors has been

TABLE II

The effect of pre-loading with saline on body weight and on the constituents of the blood and the volume of plasma

Subject Period	Weight Kg.	Serum Na mEq./L.	Serum K mEq./L.	Serum Cl mEq./L.	Serum TP Gm. %	Blood Hgb. Gm. %	Blood Hct. Vol. %	$PV_2/PV_1 \times 100$* From Hct. and Hgb. per cent	$PV_2/PV_1 \times 100$* From TP per cent
WHB									
Control	63.87	142	4.5	108	6.8	14.6	48.0	100	100
Pre-loaded	64.53	141	4.4	106	6.5	14.6	46.2	104	105
CT									
Control	72.60	142	4.4	105	7.2	15.4	49.0	100	100
Pre-loaded	73.70	140	4.2	106	6.8	13.3	46.0	123	105
AC									
Control	69.11	144	4.6	103	7.5	15.8	47.2	100	100
Pre-loaded	70.00	142	4.3	102	6.9	12.9	44.2	129	109
RD									
Control	63.90	141	4.4	103	7.0	15.2	46.0	100	100
Pre-loaded	65.08	144	4.7	107	6.8	15.3	44.1	103	103

* $PV_2/PV_1 \times 100$ indicates the calculated ratio of the new to the original plasma volume expressed as per cent. The formulae employed are:

$$1.\quad \frac{PV_2}{PV_1} = \frac{(1 - Hct_2)}{(1 - Hct_1)} \times \frac{Hgb_1}{Hgb_2}$$

$$2.\quad \frac{PV_2}{PV_1} = \frac{TP_1}{TP_2}$$

noted in connection with alcohol induced diuresis, which can be largely eliminated by giving dry salt with the alcohol and thus producing an osmotic antidiuretic stimulus (10).

In each instance when diuresis was observed in the above experiments it was associated with a decreased electrolyte concentration in the urine, a decreased creatinine U/P ratio and an unchanged rate of creatinine excretion or clearance. Urinary specific gravity fell and the rate of electrolyte excretion showed little change or moderate increase. When this type of diuretic response follows the administration of water or hypotonic solutions it is presumed to be due to diminished antidiuretic hormone secretion. It may be noted parenthetically that although direct evidence is available indicating the responsiveness of the canine neurohypophysis (or some structure lying within the distribution of the internal carotid artery) to hypertonicity (11), the effect of hypotonicity is inferential. Since no other mechanism is known which leads to precisely this type of diuresis in man, the hypothesis that the water diuresis observed in the present experiments is mediated through the supra-opticohypophyseal system seems not unreasonable.

CONCLUSIONS

1. The ingestion of one or two liters of 0.9 per cent saline solution by normally hydrated sitting subjects is not followed by a water diuresis.

2. The ingestion of one liter of 0.9 per cent saline solution by seated subjects who are on the descending limb of a previously induced water diuresis may be followed by a slight and transient augmentation of urine flow.

3. The ingestion of one liter of 0.9 per cent saline solution by normally hydrated seated subjects who have ingested excess salt on the preceding day (and whose extracellular fluid volume has presumably been expanded thereby) is followed by a prompt water diuresis. This does not occur regularly if the subjects are moderately hydropenic.

4. Responsiveness of the supra-opticohypophyseal system to changes in the volume or distribution of extracellular fluid is inferred.

ACKNOWLEDGMENT

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THE RELATIONSHIP OF THE RENAL EXCRETION OF ADRENAL CORTICOIDS TO VARIATIONS IN RENAL HEMODYNAMICS[1]

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INTRODUCTION

Measurements of urinary adrenal corticoid excretions have been widely used for the evaluation of adrenal hormone production and degradation. The possibility that renal dysfunction may alter the urinary excretion of these substances is generally ignored.

The present study was done in experimental animals in which controlled acute unilateral alterations of renal hemodynamics were produced during periods of hormone administration. The results indicate that alterations in renal hemodynamics are associated with definite changes in urinary corticoid excretion.

METHODS

Studies were done on four trained unanesthetized female dogs surgically prepared by Dr. Walter S. Kerr, Jr. These animals had their bladder trigone explanted to the anterior abdominal wall so that urine could be collected separately from each kidney. The right kidney was explanted and a skin cuff was placed around the right renal artery so that by compression of this artery the glomerular filtration rate could be unilaterally altered. The opposite kidney served as the control in these studies.

Three of the animals had no more than 10 per cent difference in creatinine and inulin clearances of the two kidneys prior to compression of the exposed renal artery. By means of varying degrees of compression of the exposed renal artery, various gradations of reduction in filtration rate were obtained unilaterally. The other animal showed a persistent decrease of approximately 50 per cent in creatinine and inulin clearances from the explanted kidney. This latter animal later had approximately 40 per cent of the control left kidney removed surgically and was restudied three weeks post-operatively.

In these studies, endogenous creatinine clearances were used as the measure of glomerular filtration rate. Urine creatinine was analyzed by the method of Bonsnes and Taussky (1) and serum creatinine by the method of Hare and Hare (2). It has been shown that the clearance of true or exogenous creatinine is identical with the inulin clearance in the dog (3). As the method used for serum creatinine chromogen has been shown to yield values equal to true creatinine (4) it was felt that the endogenous creatinine clearances represented a measure of glomerular filtration rate in these experiments. Clearances on different days of inulin and exogenous creatinine in the same animals gave values comparable to those obtained by endogenous creatinine clearances in this study.

The biologically active adrenal corticoids in the urine were measured quantitively by the Speir's mouse eosinophile bio-assay (5). Adrenalectomized BBF_1 mice were injected subcutaneously with 20 gamma of epinephrine and four hours later eosinophil counts were performed. Mice with eosinophil counts lower than 100 per cubic millimeter of whole blood were discarded. To assay urine quantitatively a standard dose-response curve was prepared using cortisone dissolved in 10 per cent ethanol. The sensitivity of this assay was found to be between 0.5 to 6 gamma of cortisone, in good agreement with Speir's results (5). Either 0.5 or 1.0 cc. of urine was injected per mouse. Four to eight mice were used per urine specimen. The urine was used either immediately or promptly frozen and used within one week of collection. The occurrence of toxic reactions was rare. The results of these urinary assays are expressed as corticoid excretion equivalent to the response obtained with a known amount of cortisone.

Normal dogs' urine consistently failed to show detectable amounts of biologically active adrenal hormones by this method. In order to obtain a positive urinary assay, it was found necessary either to stimulate adrenal activity with adrenocorticotrophic hormone (ACTH) or administer doses of 17-hydroxy, 11-dehydrocorticosterone (Compound E) or 17-hydroxycorticosterone (Compound F).

In addition to the bioassay 17, 21-dihydroxy-20-ketosteroids were measured chemically on butanol extracts of urine acidified with sulfuric acid to pH 1.0. The butanol extracts were washed with 0.1 N sodium hydroxide, 0.01 N sulfuric acid and dried with anhydrous

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TABLE I

The effects of unilateral renal artery compression on the urinary excretion of creatinine and biologically active corticoids during the intravenous administration of ACTH

Period	Time in hours		Kidney	Urine volume *cc. per period*	Creatinine conc. *mgs./cc.*	Corticoid conc. *γ/cc.*	Total creatinine excretion *mgs. per period*	Total corticoid excretion *γ per period*	Ratio creatinine excretion R/L	Ratio corticoid excretion R/L
	0	ACTH 40 mgs. I.V. started and sustained throughout experiment								
Before clamping										
I	4–5	(60 min.)	R	5.3	1.95	4	10.3	21.2	0.83	0.72
			L	7.4	1.69	4	12.5	29.6		
Slight compression right renal artery										
II	5½–6½	(60 min.)	R	5.5	1.33	2	7.3	11	0.61	0.37
			L	7.4	1.63	4	12.0	29.6		
Further compression right renal artery										
III	7–8¼	(75 min.)	R	5.0	0.86	0.5	4.33	2.5	0.28	0.05
			L	10.1	1.53	5	15.5	50.5		

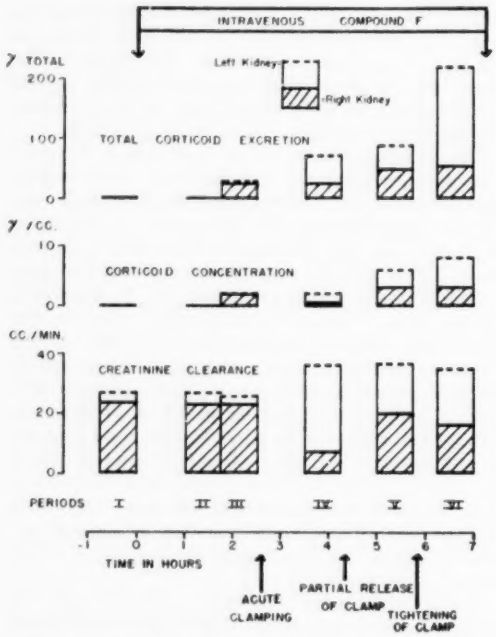

FIG. 1. THE EFFECTS OF RIGHT RENAL ARTERY COMPRESSION ON THE CREATININE CLEARANCE, URINARY CORTICOID CONCENTRATION, AND URINARY CORTICOID EXCRETION DURING THE INTRAVENOUS ADMINISTRATION OF COMPOUND F

Between periods V and VI the rate of infusion of Compound F was increased. Corticoid here refers to biologically active corticoid (see text). By total corticoid excretion is meant the total urinary excretion of biologically active corticoids per period. The periods were from 30 to 45 minutes duration.

sodium sulfate. The Porter-Silber reaction was applied directly to the butanol solution (6, 7). Solutions containing 5 and 10 micrograms per milliliter of 17-hydroxycorticosterone dissolved in butanol were used as standards. The chemical corticoids were not measured in the same experiments as the biologically active corticoids.

Methods of administration used were as follows:

(a) After an initial priming dose intramuscularly of 40 mgs. of ACTH, an intravenous infusion of 500 cc. of 5 per cent dextrose in water containing 40 mgs. o. ACTH was administered over an eight hour period. Urine collections were begun four hours after starting the intravenous infusion.

(b) 150 to 250 mgs. of cortisone acetate were administered by means of a stomach tube and urine collections were begun 90 to 120 minutes later.

(c) 50 mgs. of hydrocortisone (Compound F) [4] dissolved in 250 cc. of 2 per cent ethanol and 5 per cent dextrose were given intravenously over a six hour period and urine collections were started immediately.

RESULTS

Protocols of two representative experiments are shown in Table I and Figure 1. In the experiment recorded in Table I, positive urinary bioassays were obtained by adrenal stimulation with a continuous intravenous infusion of ACTH. The effects of unilateral compression of the right renal artery on urine volumes, creatinine and corticoid excretions in three separate periods of 60 to 75 minutes duration are shown. As the right renal

[4] This hormone was generously supplied by Merck Co., Rahway, New Jersey.

artery was progressively compressed, both creatinine and corticoid excretions diminished. In this experiment, the fall in corticoid excretion is largely due to a decrease in concentration of corticoid rather than to a change in urine volume (urine volumes on right were 5.3, 5.5, 5.0 cc. with associated corticoid excretions of 21, 11, and 2.5 gamma, respectively). Comparison of the ratios of creatinine excretion and of corticoid excretion by both kidneys revealed that corticoid excretion was more strikingly reduced by compression than was creatinine excretion.

Figure 1 illustrates the effects of the continuous intravenous infusion of Compound F and varying degrees of right renal artery compression on the creatinine clearances, urinary biologically active corticoid concentrations, and total biologically active corticoid excretions in six separate periods of 30 to 45 minutes duration. The interruptions between periods of urine collections were made in an attempt to flush out urine formed during preceding procedures from renal dead space. In period III, before renal artery compression, urinary corticoid concentrations and excretions were equal at a time when the creatinine clearances were approximately the same. The drop in creatinine clearances due to partial compression of the right renal artery was associated with decreased corticoid concentrations and total corticoid excretion from the right as compared to the left kidney. In this experiment, decreased corticoid excretion in the urine from the right kidney was related to both decreased urine volume and decreased corticoid concentration.

There is a time lag of one to two hours after beginning Compound F administration before hormone excretion became measurable. Change of hormone excretion with variation in creatinine clearance is prompt. The marked increase in corticoid excretion in the last period may be related to an increase in the rate of infusion at this time. A slight increase in the control creatinine clearances was noted three and one-half hours after starting the Compound F infusion and one

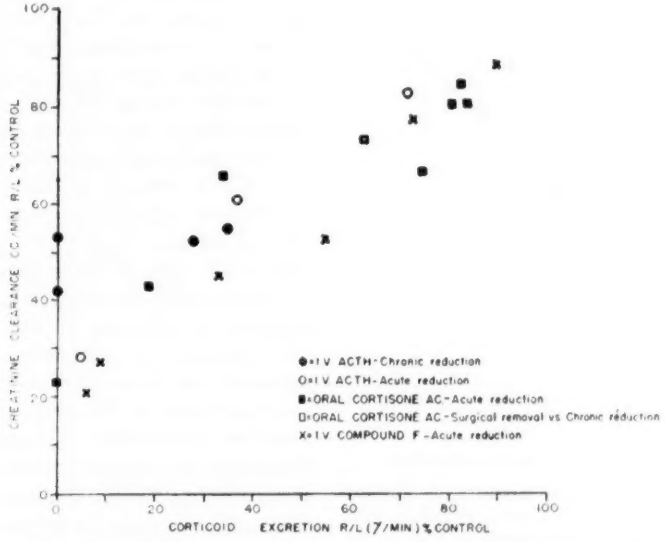

FIG. 2. A SUMMARY OF THE RESULTS OF TWENTY-ONE SEPARATE CLEARANCE PERIODS SHOWING THE RELATIONSHIP OF BIOLOGICALLY-ACTIVE CORTICOID EXCRETION TO CREATININE CLEARANCE BY THE RIGHT KIDNEY PLOTTED AS PER CENT OF THE CORRESPONDING EXCRETION BY THE CONTROL LEFT KIDNEY

Chronic reduction refers to the persistent reduction in creatinine clearance in one animal used. Surgical removal vs. chronic reduction refers to use of this animal after extirpation of 40 per cent of the control kidney (see text). The periods were 30 to 75 minutes each.

hour after partial compression of the contralateral renal artery.

Figure 2 is a summary of the results of twenty-one experimental periods, including forty-two urinary bio-assays. There is a positive correlation between creatinine clearance and biologically active corticoid excretion. In no instance did biologically active corticoid excretion from the right kidney exceed that from the control kidney on the left. The three points on the extreme left representing a zero corticoid excretion may simply be a reflection of the lower limit of sensitivity of the bio-assay method, and therefore cannot be further interpreted. However, a line which best fits the observed data would intersect the ordinate significantly above zero. This suggests that at very low rates of glomerular filtration the urinary excretion of biologically active adrenal corticoids decreases sharply.

Figure 3 summarizes the results of 20 additional experimental periods in which the chemical corticoid excretions were measured. A linear relationship between creatinine clearance and chemical corticoid excretion is apparent. A line which best fits the observed data would pass through zero.

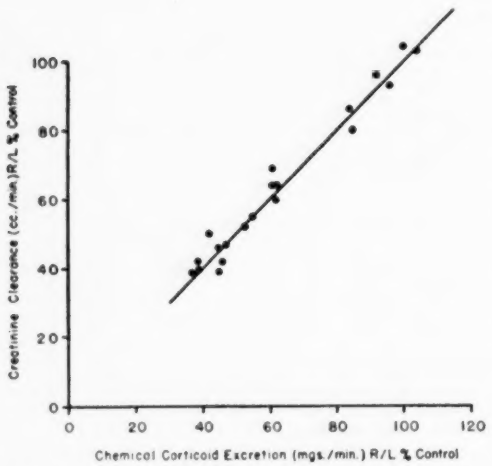

FIG. 3. A SUMMARY OF THE RESULTS OF TWENTY SEPARATE CLEARANCE PERIODS SHOWING THE RELATIONSHIP OF CHEMICAL CORTICOID EXCRETION TO CREATININE CLEARANCE BY THE RIGHT KIDNEY PLOTTED AS PER CENT OF THE CORRESPONDING EXCRETION BY THE CONTROL LEFT KIDNEY

Cortisone acetate was administered by stomach tube to obtain the observed levels of hormone excretion in all these periods. The periods were 20 to 60 minutes each. The line drawn arbitrarily through the points has a slope of 1.0.

DISCUSSION

By compressing one renal artery and comparing the excretion of hormone from that kidney with that of the opposite control kidney, it was possible to assess the relative effects of decreased filtration rate or blood flow on the excretion of biologically active corticoids without knowledge of the blood levels of these hormones. It has, however, been recently shown that following the administration of ACTH or cortisone intravenously or cortisone acetate orally, the blood level of 17-hydroxycorticosteroids rises two to ten times above its original level (8, 9, 10) and with the known values for renal blood flow, the minute quantities of hormone excreted in these experiments must represent only a small fraction of the hormone perfusing kidneys. This is in agreement with the absence of a significant arterial-venous concentration difference of 17-hydroxycorticosteroids in the dog kidney noted by Nelson (9), and the low estimate of renal clearance of formaldehydogenic corticosteroids made by Corcoran and Page (11).

From the manner in which these experiments were conducted, it is impossible to state whether the observed acute changes in hormone excretion were due to changes in filtration rate or to the simultaneous alterations occurring in renal blood flow, or tubular function. Goldman and Bassett (12) noted that the excretion of neutral lipid-soluble reducing steroids was proportional to the excretion of creatinine in their study of patients with cirrhosis and congestive heart failure and suggested that the excretion of corticosteroids may be proportional to the filtration rate. As no information is available at present regarding the filtrability of adrenal corticoids through the glomerular membrane, it is not possible to decide whether the changes in hormone excretion were due to changes in the amount filtered, or in tubular reabsorption or tubular secretion or hormone.

The disappearance of biologically active corticoids from the urine at low levels of glomerular filtration rate in contrast to the continued excretion of chemical corticoids may simply be an artifact arising from the limit of sensitivity of the

bio-assay used. Another cause for this apparent difference may be that the high rates of chemical corticoid excretion in these experiments were masking an increment of tubular reabsorption of chemical corticoids. The possibility exists, however, that at a given filtration rate proportionately more of the filtered corticoids which are biologically active are reabsorbed by the renal tubules than is the case with the majority of corticoids measured chemically.

SUMMARY

The effects of acute variations in glomerular filtration rate on the renal excretion of biologically active adrenal corticoids and chemical corticoids were evaluated in dogs. Acute alterations in renal hemodynamics were produced by unilateral compression of one renal artery during periods of adrenal stimulation or administration of adrenal corticoids. With reduction of glomerular filtration rate there occurred a decrease in excretion of both biologically-active adrenal corticoids and chemical corticoids.

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THE NORMAL LEVELS OF 17-HYDROXYCORTICOSTEROIDS IN THE PERIPHERAL BLOOD OF MAN [1]

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(Submitted for publication April 21, 1953; accepted May 15, 1953)

Research in the field of adrenal cortical function has been limited by the inability of existing techniques to measure precisely the kind and amounts of steroids elaborated by the gland. Particularly in man, the indicators have been indirect and inadequate. These have consisted principally of the alterations in certain non-steroid constituents of the blood, changes in the total number of circulating lymphocytes and eosinophils, and variations in end products of steroid metabolism found in the urine.

Biological assays (1, 2) of the blood level of adrenal cortical hormones were developed with the hope that they would provide greater specificity, but have proved to be neither sufficiently sensitive nor precise. Chemical methods (3, 4, 5) for the determination of blood steroids have been proposed.

The first satisfactory direct quantitative measurement of 17-hydroxycorticosteroids in the blood of human subjects was reported by Nelson and Samuels (6). This would include such "17-OH" compounds as E, F, and S, when present. Actually, the major component in the peripheral blood of man appears to be 17-hydroxycorticosterone (compound F) (6, 7, 8, 9). Since this seems to be the principal 17-hydroxycorticosteroid secreted by the normal adrenal gland (9), the method offers an excellent direct measure of adrenal cortical activity.

This report is concerned with the concentration of circulating 17-hydroxycorticosteroids in the peripheral venous blood of normal adults, the range of normal values, diurnal variations, and the degree of fluctuation in blood levels of cortical steroids for any one individual from day to day and week to week.

MATERIALS AND METHODS

The levels of 17-hydroxycorticosteroids in plasma were determined by the method of Nelson and Samuels (6). A few samples were run by both this method and that of Sweat and Farrell (10). Subjects were medical students, residents, graduate students and hospital personnel having no known diseases. The only exceptions to this were the aged patients, none of whom were acutely ill, although the majority suffered from the usual degenerative changes of old age.

Blood samples were obtained by venipuncture and placed in tubes containing 4 mg. of heparin. These were then centrifuged, the plasma removed and the samples refrigerated. All specimens, save for the diurnal ones, were drawn between 8 and 8:30 A.M. on fasting subjects. In the case of diurnal studies, some subjects were studied while fasting; others were permitted to eat. These will be designated.

RESULTS

1. *Comparison of analytical methods*

The results on duplicate samples analyzed by the technique of Nelson and Samuels, and that of Sweat and Farrell are given in Table I. It is evident that the two procedures measure the same compounds, since the values are almost identical.

[1] This investigation was supported in part by research grants A-29 from the National Institute of Arthritis and Metabolic Diseases and M-451 from the National Institute of Mental Health, of the National Institutes of Health, Public Health Service, in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-136, in part under a research grant from the American Cancer Society and in part by the Scottish Rite Foundation.

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[4] Postdoctoral Fulbright Travel Fellow and Baxter Research Fellow.

TABLE I

Comparison of analytic methods

Subject	Nelson and Samuels Technique 17-OH Corticosteroids *μg./100 ml. plasma*	Sweat and Farrell Technique 17-OH Corticosterone *μg./100 ml. plasma*
B	30	28
L	11	13
C	11	10
	20*	20*
	35*	31*
D	16	16
Ad	25*	31*
A	27*	27*
	30*	30*
W	20*	25*

* Levels after ACTH administration.

2. Normal values—Young healthy adults

Two hundred and sixty-seven determinations were made on 120 adults, 91 of whom were male and 29 female. The age range was 20 to 45. In Figure 1, the distribution of normal values is charted. Since the distribution of normal values was skewed, standard statistical techniques were not applicable. The curve was transformed into a normal distribution, therefore, by converting all values to the 2/3 power ($\times$ 2/3). The means, standard deviations, standard errors of the means and 95 per cent range were then calculated by routine methods. These values were then reconverted to give actual concentrations.

As indicated in Table II, the mean of the group is 13 μg. per 100 ml. plasma with a standard deviation of ± 6 μg. Values ranged from less than 2 μg. to 34 μg. per 100 ml. plasma. Since each value represents a single measurement, and as no results were excluded, the data must include analytical errors, as well as human variation. In view of this, it is reasonable to accept the 95 per cent range, 3–26 μg. per 100 ml. of plasma, as representing the normal distribution. It should be emphasized that these are fasting values and reflect steroid levels between 8 and 8:30 A.M.

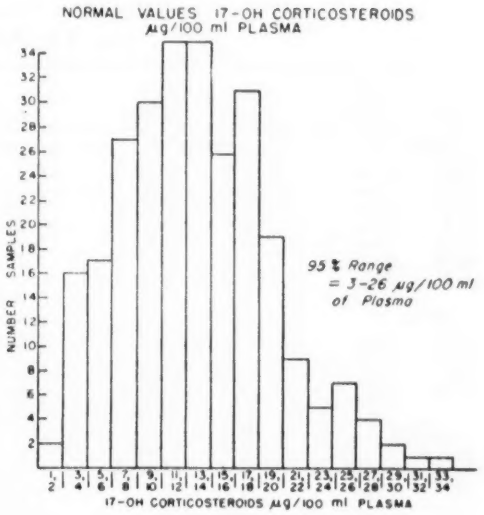

FIGURE 1

The mean value for females was 15 ± 6 μg. per 100 ml. plasma compared to 12 ± 6 μg. per 100 ml. plasma for males. A sex difference, if any exists, must be small.

3. Normal values—Aged population

A group of 29 elderly patients, 27 males and 2 females, whose average age was 78 years, was also studied. The mean steroid level for the group was 12 ± 6 μg. per 100 ml. plasma (Table II).

4. Range of variation in the same individual

Twenty-one subjects were studied repeatedly over a period of several months. Blood samples were drawn at intervals varying from several days to several weeks. There was considerable variation in steroid levels from day to day and week to week in most individuals (Table III). Examples are: Subject L., 6 μg. to 18 μg.; Subject

TABLE II

Normal values—17-hydroxycorticosteroids μg. per 100 ml. plasma

Groups	No. of subjects	No. of studies	Age	Mean and S.D.	Range	Mean and S.E.
Young adults	120	267	20–45	13 ± 6 μg.	2 to 34 μg.	13 ± 0.4
Males	91	195	20–45	12 ± 6 μg.	2 to 34 μg.	12 ± 0.4
Females	29	72	20–45	15 ± 6 μg.	2 to 31 μg.	15 ± 0.7
Aged	29	29	64–95	12 ± 6 μg.	3 to 24 μg.	12 ± 1.0

TABLE III

Range of variation in same individual

Date	17-OH corticosteroids	Date	17-OH corticosteroids	Date	17-OH corticosteroids	Date	17-OH corticosteroids	Date	17-OH corticosteroids
*Subject A**		*Subject F**		*Subject J*		2/15	9 μg.	*Subject Q*	
2/29	17 μg.	2/21	18 μg.	4/7	27 μg.	3/20	19 μg.	1/14	11 μg.
3/10	14 μg.	2/26	20 μg.	4/11	17 μg.	4/11	10 μg.	3/19	19 μg.
3/21	12 μg.	3/11	20 μg.	4/18	21 μg.	4/22	18 μg.	4/12	3 μg.
4/2	13 μg.	3/25	26 μg.	4/25	14 μg.	5/1	16 μg.	5/20	16 μg.
4/11	13 μg.	4/1	25 μg.	5/14	7 μg.			5/27	22 μg.
4/30	12 μg.	4/16	13 μg.	7/2	13 μg.	*Subject N*		6/2	21 μg.
6/4	19 μg.	4/22	8 μg.			12/27	2 μg.		
		5/5	19 μg.	*Subject K*		1/10	8 μg.	*Subject R*	
*Subject B**				2/13	14 μg.	1/20	12 μg.	10/30	11 μg.
2/21	19 μg.	*Subject G*		2/18	34 μg.	2/7	11 μg.	11/1	6 μg.
2/28	31 μg.	2/20	21 μg.	2/20	18 μg.	2/19	13 μg.	11/5	5 μg.
3/6	14 μg.	2/27	21 μg.	2/25	27 μg.	4/12	8 μg.	11/8	18 μg.
3/20	14 μg.	3/7	15 μg.	2/27	23 μg.	5/16	8 μg.	12/4	16 μg.
		3/14	8 μg.	2/28	20 μg.	6/2	11 μg.	1/31	14 μg.
Subject C		3/22	10 μg.	3/3	12 μg.	6/19	13 μg.	2/14	10 μg.
2/21	19 μg.	5/22	24 μg.	3/7	23 μg.			2/22	18 μg.
2/28	25 μg.					*Subject O*		3/1	18 μg.
3/7	13 μg.	*Subject H**		*Subject L*		1/10	11 μg.		
3/13	29 μg.	2/20	6 μg.	2/20	9 μg.	2/17	16 μg.	*Subject S*	
		2/27	13 μg.	2/22	15 μg.	3/1	5 μg.	7/12	11 μg.
*Subject D**		3/12	6 μg.	2/25	10 μg.	3/24	11 μg.	8/1	12 μg.
2/18	17 μg.	3/25	18 μg.	2/29	13 μg.	4/12	19 μg.	8/15	8 μg.
2/25	26 μg.	4/1	26 μg.	3/3	18 μg.	4/26	3 μg.	8/20	8 μg.
3/3	23 μg.	4/10	14 μg.	3/7	10 μg.	7/9	12 μg.	11/1	10 μg.
3/10	13 μg.	4/17	16 μg.	3/10	11 μg.	7/29	21 μg.		
4/20	16 μg.	6/19	8 μg.	3/12	8 μg.	8/12	20 μg.	*Subject T*	
		7/1	14 μg.	3/14	6 μg.	9/11	12 μg.	4/5	15 μg.
*Subject E**				3/17	10 μg.	9/15	20 μg.	5/12	2 μg.
2/18	19 μg.	*Subject I**		3/19	6 μg.			6/18	13 μg.
2/26	19 μg.	4/4	19 μg.	3/21	9 μg.	*Subject P*		6/25	10 μg.
3/4	30 μg.	4/10	28 μg.	6/6	17 μg.	1/10	11 μg.		
3/11	19 μg.	4/17	15 μg.			1/14	19 μg.	*Subject U*	
4/2	17 μg.	5/7	22 μg.	*Subject M*		2/11	10 μg.	11/20	9 μg.
4/8	26 μg.	5/13	12 μg.	12/8	8 μg.	5/17	17 μg.	11/26	26 μg.
4/14	13 μg.	7/11	12 μg.	12/22	11 μg.	5/29	14 μg.	11/29	8 μg.
7/1	18 μg.			1/4	3 μg.	6/10	8 μg.	12/3	8 μg.
7/9	19 μg.			1/12	17 μg.	7/9	10 μg.		

* Female; all other subjects male.

K., 12 μg. to 34 μg.; Subject H., 6 μg. to 26 μg., and Subject N., 3 μg. to 21 μg. per 100 ml. plasma.

5. *Diurnal variations*

These data are listed in Table IV. Observations on the fasting and non-fasting subjects were pooled since the mean values on those who ate breakfasts and those who did not were almost identical.

Although there were a number of individual exceptions, in general the tendency was for the highest levels to occur in the early morning. Between 8 A.M. and 12 noon the level tends to drop. As the day advances, steroid levels fluctuate considerably, but continue to remain on this lower plateau (Figure 2).

DISCUSSION

In the normal, healthy, fasting adult, the mean plasma level of 17-hydroxycorticosteroids was 13 ± 6 μg. per 100 ml. between 8 and 8:30 A.M. Values for males and females were not significantly different. Since almost identical values were found in the 26 elderly subjects whose average age was 78, it seems likely that adults maintain virtually the same range of peripheral blood adrenal steroid levels over the span of their mature years.

For any one individual, values were found scattered throughout the normal range when studies at 8 A.M. were repeated at daily, weekly, or monthly intervals. This range is much wider than that of most chemical constituents of the

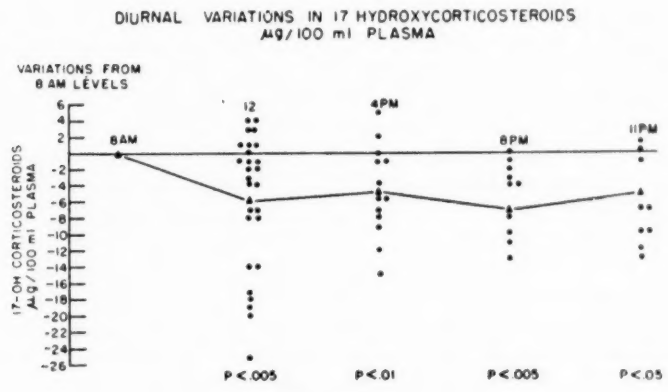

FIGURE 2

TABLE IV

Data-diurnal variations in 17-hydroxycorticosteroids

Subjects	8 A.M.	9	10	11	12 Noon	1 P.M.	2	3	4	5	6	7	8	9	10	11	12 Midnight
No. 1	16			11						15						16	
No. 2	16			13						16					16		
No. 3	14				6				2				4				4
No. 4	12			10					7								
No. 5	19	13			23				11								
No. 6	9	4			2		8		5								
No. 7	23	12		6	3		5		8								
No. 8	12	12			2				13								
No. 9	8				4				10								
No. 10	7	2			10					8							
No. 11	15				15				14				8			3	
No. 12	10				11				4				6			9	
No. 13	15				16				6				2			2	
No. 14	9								9				7			2	
No. 15	8					7			13				5			9	
No. 16	11					7			10				7			18	
No. 17	11					6			5				3			4	
No. 18	13					11			6				2			3	
No. 19*	27	14	9		8												
No. 20*	20	22	14		6												
No. 21*	26	25	20		23												
No. 22*	14	12	20		18												
No. 23*	25	21	17		17												
No. 24*	30	21	8														
No. 25*	26	17	11		8												
No. 26*	23	22	12		22												
No. 27*	29	20	18		15												
No. 28*	31	20	10		6												
No. 29*	13	6	6		12												
No. 30*	11				12												
No. 31*	8				6												
No. 32*	9				7												
No. 33*	8				7												
No. 34*	17				10												
No. 35*	11				14												
No. 36*	27				10												
No. 37*	16				12												

* Fasting—no breakfast.

circulating fluids under normal conditions, and argues against a simple homeostatic concept. It must be recognized, however, that the extraction procedures may remove steroid which is loosely bound as well as that which is free. Until this problem is answered, it is impossible to draw conclusions regarding the constancy of the *free* 17-hydroxycorticosteroid levels.

While the spread in normal individuals may be wide, disturbances of the adrenal cortex which can be diagnosed clinically usually give values outside this range. In most Addisonians there is no circulating 17-hydroxycorticosteroids in the peripheral blood; although a few who presumably have remnants of intact tissue show low levels (11). Patients with Cushing's disease have pathologically elevated titres (9, 11).

Diurnal studies demonstrated certain trends, although there were many individual exceptions. The most constant finding was the presence of maximal 17-hydroxycorticosteroid levels in the early morning. Only rarely were later samples higher, and then only by small amounts. Whether this high level early in the morning represents an accumulation of steroids during sleep, a greater need and increased steroid production in the early morning hours, or an increase in steroid production with a decrease in peripheral utilization it is difficult to say. In any event, most of our subjects demonstrated a decline in 17-hydroxycorticosteroid levels during the day. Late evening levels remained low. This is consistent with the data of Pincus, Hoagland, Romanoff, and Carlo (12, 13), who found comparable changes in the diurnal excretion of 17-ketosteroids and neutral reducing lipids in the urine, as well as those of Sandberg and his co-workers (14), who observed the same pattern in the urinary 17-hydroxycorticosteroids following glucuronidase hydrolysis. Both groups recorded the highest urinary levels in the morning.

SUMMARY

1. Plasma levels of 17-hydroxycorticosteroids were determined in normal adult subjects by the method of Nelson and Samuels.

2. The mean 17-hydroxycorticosteroid level in young adults was 13 ± 6 μg.; 15 ± 6 μg. for females and 13 ± 6 μg. for males. The 95 per cent range for the group was 3–26 μg. per 100 ml. of plasma.

3. In a group of elderly subjects whose mean age was 78 years, the average level was 12 ± 6 μg.

4. Diurnal studies revealed high 17-hydroxycorticosteroid levels in the early morning. These tended to drop to a lower plateau over the course of the day and evening, although considerable individual variation was evident.

5. The wide range of normal 17-hydroxycorticosteroid values suggests an adrenal cortical homeostatic balance in man which is less sensitive than that for most chemical constituents in the body fluids.

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ARTERIAL BLOOD GASES AND ACID–BASE BALANCE IN NORMAL CHILDREN [1]

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Robinson (1) studied the effect of age on the arterial blood gases and the acid-base balance of normal males from 5 to 91 years of age. In addition to the well known increase in oxygen combining capacity which occurs during childhood and adolescence, Robinson found moderately lower mean values for hemoglobin saturation in adults above 40 years of age, and slightly higher mean values for arterial CO_2 content and CO_2 tension in adults as compared with children. In the case of the alkaline reserve, mean values varied between 21.2 and 22.2 millimols per liter for all age groups except the youngest. Robinson found unusually low values of alkaline reserve in children 4 to 6 years of age. The present study was undertaken to supplement the work of Robinson in the years of childhood and adolescence, in order to establish more firmly the range of variation that may be expected in healthy children, and to provide a basis for the study of arterial blood gases and acid base balance in disease.

MATERIAL

The subjects of the study were drawn from three sources:

1. Children of 10 years and younger were of both sexes. They had been carefully selected from patients at Bobs Roberts Memorial Hospital as representing physiologically normal states at the time the blood samples were drawn. Breakfast was withheld and the child was kept in bed until after the sample was taken.

2. Subjects between 11 and 17 years of age were normal healthy boys [3] who came to the laboratory to participate in various tests for physical fitness. The results of most of these tests have been described earlier (2–5). The boys came to the laboratory in the early morning, without breakfast, and had been resting in the recumbent position for approximately two hours before the arterial blood sample was drawn.

3. Nine male medical students were also included in the study. Blood samples were drawn approximately two hours after breakfast and after rest in the recumbent position for 15 to 30 minutes.

METHODS

Arterial samples were drawn from the brachial artery in the adults and older children and from the femoral artery in younger children. They were drawn without local anesthetic and usually with little discomfort to the subject; however, breathholding could not always be avoided, and in the younger children crying usually occurred.

The samples were drawn into syringes whose dead space was filled with heavy mineral oil. The blood was transferred immediately, either under oil into centrifuge tubes containing 1 mg. heparin (Connaught Laboratories) dissolved in 0.02 ml. saline, thence, without contact with air, into sampling tubes containing mercury; or directly into sampling tubes containing 0.02 ml. of the heparin-saline solution above the mercury. The latter technique was used in the later studies which included most of the adults and children under 11 years of age. The samples were kept in ice water in a refrigerator until analysis was completed.

The oxygen and carbon dioxide contents of the arterial blood samples were determined by the manometric method of Van Slyke and Neill (6), using 0.5 ml. samples. The determinations were performed in duplicate, or until good checks were obtained. The oxygen and carbon dioxide contents were also determined after the blood sample had been equilibrated for 20 minutes at 37° C. in tonometers of approximately 325 ml. capacity which contained carbon dioxide at an approximate tension of 40 mm. Hg and oxygen at 185 mm. Hg. Oxyhemoglobin (HbO_2) content and capacity were determined by subtracting the appropriate amount for physically dissolved oxygen from the oxygen content of the blood as drawn and of the blood after equilibration respectively. Percentage oxygen saturation was calculated from HbO_2 content and HbO_2 capacity. No correction was made for the errors in oxygen capacity measurements which Roughton, Darling and Root (7) have shown may be introduced by the tonometer method. From the blood data and the gas contents of the tonometer, determined by Haldane analy-

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[3] We are indebted to the University of Chicago Settlement House, the Valentine Boys Club, the Hyde Park Neighborhood House and friends for their kindness and interest in sending these boys to the laboratory for the tests.

sis, alkaline reserve (T_{40}) of blood and serum, CO_2 tension (pCO_2), and serum bicarbonate concentration ($BHCO_3$)$_s$ were calculated by methods developed at the Harvard Fatigue Laboratory (8). The pH_s was calculated using the Henderson-Hasselbalch equation.

In 64 older children, 10 to 17 years of age, additional analyses were made in order to describe the acid-base or electrolyte balance of the plasma more completely. These included plasma protein, chloride, phosphorus, lactic acid and sodium. The methods used were: for protein, determination of total nitrogen by a modification of the micro Kjeldahl method described by Ma and Zuazaga (9) and calculation as protein by multiplying by the factor 6.25 (after correction for non-protein nitrogen); for chloride, the silver iodatethiosulfate titrimetric method of Sendroy (10), using 0.1 ml. samples; for phosphorus, an adaptation of the method of Fiske and Subbarrow (11) to 0.2 ml. samples, with the aid of the Evelyn colorimeter; for lactic acid, a modification by Edwards (12) of the method of Friedemann, Cotonio and Shaffer (13), using 0.5 ml. of whole blood; for sodium, a modification by Consolazio and Dill (14) of the method of Butler and Tuthill (15). The base equivalence of protein, or proteinate ion concentration, was calculated from the equation of Van Slyke, Hastings, Hiller and Sendroy (16), milliequivalents of base bound by protein = $0.104 \times$ grams protein $\times$ (pH_s—5.08), and of phosphate, by assuming a valence of 1.8.

RESULTS

After plotting the individual results against age, as in Figure 1, the ages which showed a similar range of variation were grouped together and the mean and standard deviation of each group was determined. The significance of differences between the means of successive groups was determined by Fisher's test. The results are given in Table I. The ranges of normal variation, defined as ± 2 standard deviations from the mean, are shown graphically in Figure 1. Several age groupings which did not prove to be significantly different from preceding or following groups were retained because the narrower ranges of variation secured for some groups by this means appeared to define better the range of normal variation.

The results indicate that HbO_2 capacity varies little with age between 3 and 14 years. Above 14 years the HbO_2 capacity level rises; the mean value of 19.7 vol. per cent for boys of 15 to 17 years is, however, significantly below the mean of 21.2 vol. per cent which was found for the adult group of this study.

The arterial blood of younger children appears to be slightly less saturated with oxygen than that of older children and adults. A mean percentage saturation of 94.2 per cent for children of 2 to 4 years is significantly lower than that of 95.8 per cent for children of 7 to 17 years ($t = 3.5$); the mean of 95.3 per cent for children of 5 and 6 years lies between those of the younger and older groups. The lower mean value for the youngest group is due chiefly to values of approximately 92 per cent in three children of 3 and 4 years, and to the fact that the younger children did not show saturation levels above 97 per cent, such as were observed frequently in the older children. Values for boys of 14, 15 and 16 years varied more widely than those for younger children and adults.

In the case of arterial blood CO_2 content (CO_2)$_b$ and serum bicarbonate ($BHCO_3$)$_s$ differences between age groups above 6 years did not prove to be significant, but the groupings were retained in order to represent adequately the trend toward higher values which begins after 10 years. Analysis of data for the children between 10 and 17 years gave correlation coefficients of 0.306 for (CO_2)$_b$ with age, and 0.398 for ($BHCO_3$)$_s$ with age. These values are significant at better than the 1 per cent level.

Values of alkaline reserve of the blood (T_{40})$_b$ show no definite trend with age between 7 and 17 years, but a mean of 46.4 vol. per cent for this age group is significantly lower than that for the adults of this study, 47.9 vol. per cent. With the exception of three values which are low, children of 4 to 6 years gave values of (T_{40})$_b$ which approximate those of older children, but children of 2 and 3 years had much lower values as a rule. With the exception of one high value of 49.2 vol. per cent, (T_{40})$_b$ ranged between 34.9 and 43.3 vol. per cent in these young children.

The same trends with age that characterize (T_{40})$_b$ values apply in general to corresponding (T_{40})$_s$ values. However, in the case of (T_{40})$_s$ a trend toward higher values occurred at 14 years. A difference of 1.1 vol. per cent between the means of the 7 to 13 year and the 14 to 17 year groups is probably significant. This trend appears to have been masked in the case of (T_{40})$_b$ by the higher red cell volume of the older group, for the CO_2 concentration in the cells is lower than that in the serum.

The pCO_2 of arterial blood shows wide variation in our data. A mean pCO_2 of 41.3 mm. Hg

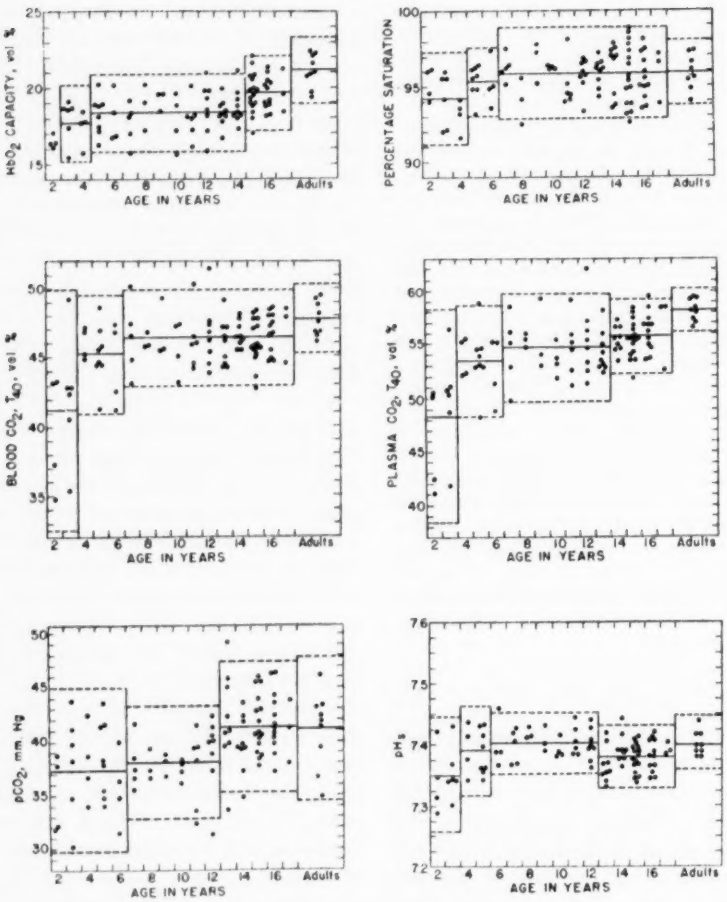
HbO_2 CAPACITY, vol %
PERCENTAGE SATURATION
BLOOD CO_2 T_{40}, vol %
PLASMA CO_2 T_{40}, vol %
pCO_2, mm. Hg
pH_a
AGE IN YEARS
2 4 6 8 10 12 14 16 Adults

FIG. 1. RELATION OF AGE TO THE BLOOD GAS CONCENTRATIONS AND THE ACID-BASE EQUILIBRIUM IN THE ARTERIAL BLOOD OF NORMAL CHILDREN

Data for each child are plotted according to the age of the child. The solid horizontal lines represent the mean value for age groups which show similar trends. The broken lines inclose the range of normal variation as defined by ± 2 standard deviations from the mean of each group. Data for 9 young male adults are included for comparison. Blood CO_2 T_{40}, or alkaline reserve, is defined as the CO_2 content of fully oxygenated blood which has been equilibrated at 37° C. with excess O_2 and with CO_2 at a tension of 40 mm. Hg. Plasma CO_2 T_{40} was calculated from blood CO_2 T_{40}, using a line chart (8) which takes O_2 capacity into acount.

for children of 13 to 17 years is very close to that of the adults, 41.1 mm. Hg. It is significantly higher than the mean values for younger children, 37.3 mm. Hg for children 1 to 6 years old and 38.0 mm. Hg for those of 7 to 12 years old.

With few exceptions the arterial pH_a values found in this study fall within the limits generally accepted as normal, 7.35 to 7.45. The 2 to 3 year group, with a mean pH_a of 7.35 and with three values below 7.32, constitutes a notable exception. In the other age groups variations in distribution occur, with the 4 to 5 year and the 13 to 17 year groups tending to show lower pH_a values than both the children of 7 to 12 years and the adults. The mean pH_a of 7.38 for boys of 13 to 17 years is significantly lower than the mean of 7.40 for the children of 6 to 12 years and the adults. It is puzzling to note that Robinson (1)

found a higher pH_a value for adolescents than for other age groups of his study.

The acid-base balances for the various age groups of our study are represented graphically in Figure 2 in which $(BHCO_3)_a$, pCO_2 and pH_a are plotted simultaneously by means of triaxial coordinates, according to the method of Hastings and Steinhaus (17) and of Shock and Hastings (18). In such charts the range of normal variation is customarily represented by a regular hexagon in the center. In the charts of Figure 2 the center represents the mean for 21 normal adults. This number includes the 9 adults of our study and 12 adult males studied by Dill, Edwards and Consolazio (19) by the same methods. The irregular hexagon around the center is bounded by lines which represent ± 2 standard deviations from the mean $(BHCO_3)_a$, pCO_2 and pH_a of the 21 normal adults. Thus the results for each age group may be compared readily with the acid-base balance of the normal adult.

In the youngest age group, 2 to 3 years, the displacement of the acid-base balance from the adult pattern is almost entirely in the direction of fixed acid excess or metabolic acidosis. In both the 4 to 6 year group and the 7 to 11 year group there is still evidence of metabolic acidosis as judged by adult standards, but to a lesser degree than in the very young children. The acid-base pattern in these groups, however, tends to be shifted to the right in the direction of CO_2 deficit or respiratory alkalosis, the net effect producing a normal pH_a, but often a slightly low $(BHCO_3)_a$ and pCO_2. As age increases beyond 11 years the

TABLE I

Effect of age upon the blood-gas concentrations and acid-base equilibrium of arterial blood

Age range	No. of cases	HbO_2 capacity Mean	S.D.	t*	Age range	No. of cases	Saturation Mean	S.D.	t*
		vol. %	*vol. %*				*per cent*	*per cent*	
1.5– 2.4	4	16.5	—	—	1.5– 4.4	14	94.2	1.56	—
2.5– 4.4	10	17.7	1.26	—	4.5– 6.4	12	95.3	1.17	2.0
4.5–14.4	59	18.4	1.29	1.5	6.5–17.4	78	95.8	1.51	1.0
14.5–17.4	31	19.7	1.21	4.7	Adults	9	95.9	1.07	0.2
Adults	9	21.2	1.09	3.3					
		Blood CO_2 content					$(BHCO_3)_a$		
		vol. %	*vol. %*				*mEq./L.*	*mEq./L.*	
1.5– 3.4	10	40.1	5.58	—	1.5– 3.4	10	20.0	2.55	—
3.5– 6.4	16	44.4	2.74	2.6	3.5– 6.4	16	22.5	1.34	3.3
6.5–11.4	21	45.5	2.49	1.3	6.5–11.4	21	23.1	1.21	1.5
11.5–14.4	26	46.8	2.51	1.8	11.5–13.4	17	23.7	1.54	1.4
14.5–17.4	31	47.5	2.07	1.3	13.5–15.4	25	24.0	0.91	0.8
Adults	9	48.5	2.41	1.2	15.5–17.4	15	24.6	1.16	1.9
					Adults	9	25.2	0.96	1.2
		Blood T_{40}†					Plasma T_{40}†		
		vol. %	*vol. %*				*vol. %*	*vol. %*	
1.5– 3.4	10	41.2	4.33	—	1.5– 3.4	10	48.4	4.96	—
3.5– 6.4	16	45.2	2.14	3.2	3.5– 6.4	16	53.5	2.60	3.5
6.5–17.4	78	46.4	1.71	2.3	6.5–13.4	38	54.7	2.52	1.6
Adults	9	47.7	1.29	2.2	13.5–17.4	40	55.8	1.80	2.1
					Adults	9	58.2	1.05	3.9
		pCO_2					pH_a		
		mm. Hg	*mm. Hg*						
1.5– 6.4	26	37.3	3.82	—	1.5– 3.4	10	7.35	0.047	—
6.5–12.4	29	38.0	2.62	0.8	3.5– 5.4	12	7.39	0.037	2.3
12.5–17.4	49	41.3	3.05	4.9	5.5–12.4	33	7.40	0.025	1.4
Adults	9	41.1	3.34	—	12.5–17.4	57	7.38	0.026	3.8
					Adults	9	7.40	0.020	—

* The t score measures the significance of the difference between a given mean and the mean immediately preceding.

† T_{40}, or alkaline reserve of the blood, is defined as the CO_2 content of oxygenated blood equilibrated at 37° at a CO_2 tension of 40 mm. Hg. T_{40} of the plasma was calculated from blood T_{40}, using a line chart which takes into account $rHCO_3$ and O_2 capacity (8).

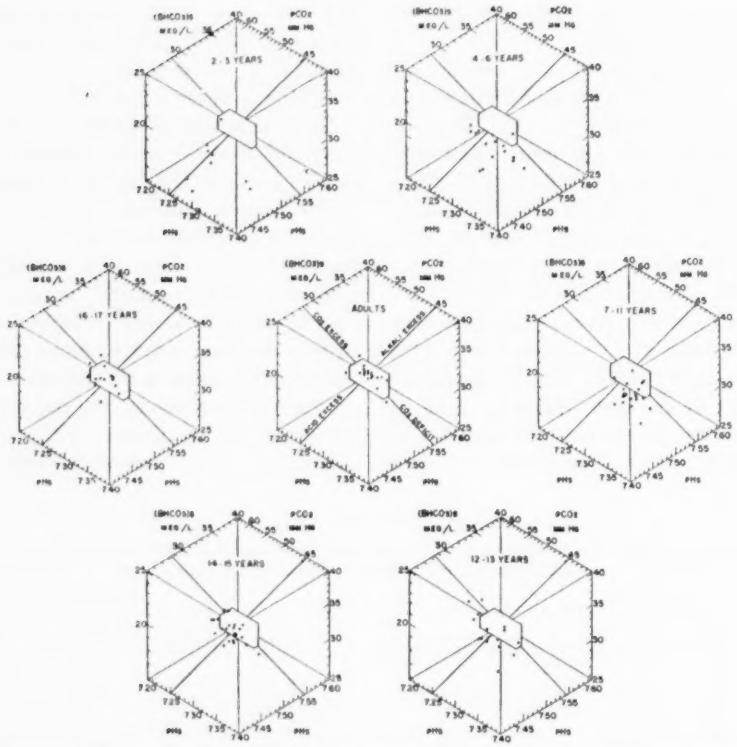

FIG. 2. SERUM BICARBONATE, CO_2 TENSION, AND pH_a IN THE ARTERIAL BLOOD OF CHILDREN OF VARIOUS AGE GROUPS

The data are plotted on triaxial coordinates according to the method of Hastings and Steinhaus (17). In the adult group the values represented by open triangles were taken from a study of the arterial blood of 12 normal adults by Dill, Edwards and Consolazio (19). The hexagon in the center of each chart incloses the area of normal variation for adults as defined by ± 2 standard deviations from the mean serum bicarbonate, pCO_2 and pH_a for 21 normal male adults, 9 from the present study and 12 from the study of Dill, Edwards, and Consolazio (19).

TABLE II

*Serum electrolyte concentrations in the arterial blood of normal boys 10 to 17 years of age**

Age	No. of cases	Bicarbonate	Chloride	Proteinate	$HCO_3^- + Cl^- + Prot.^-$	Phosphate	Lactate	Sodium
yr.		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*
10–12	11	23.0 (20.7–24.9)	104.2 (99.4–107.6)	16.7 (14.6–19.4)	143.9 (141.3–146.0)	2.55 (10) (2.25–3.05)	1.5 (0.6–2.1)	141.1 (8) (136.4–143.8)
13–14	20	23.7 (21.6–26.0)	104.3 (101.4–107.6)	16.7 (14.9–18.3)	144.7 (142.7–146.8)	2.61 (12) (2.10–3.05)	1.6 (0.9–2.8)	140.3 (12) (136.2–143.4)
15–17	33	24.3 (22.2–27.0)	104.3 (100.0–107.5)	17.2 (15.4–19.0)	145.9 (142.4–149.9)	2.45 (29) (1.91–3.07)	1.5 (0.9–2.8)	141.0 (24) (136.6–145.5)
Adult†	12	25.1 (23.9–26.0)	104.6 (103.7–105.7)	17.4 (15.9–18.3)	147.1 (144.8–149.6)		1.4 (1.2–1.9)	140.0 (131.9–143.1)

* The upper value in each case represents the mean, and quantities below in parentheses, the range of variation. In the case of phosphate and sodium, numbers in parentheses to the right of the mean indicate the number of cases included in the mean.

† Data taken from a study by Dill, Edwards and Consolazio (19).

degree of metabolic acidosis decreases, and many of the cases in the 12 to 13 year and 14 to 15 year groups fall within the normal range of variation. Their values, however, tend to be shifted farther to the left, in the direction of CO_2 excess. At 16 to 17 years the adult pattern of acid-base equilibrium appears to be fairly well established, although a tendency toward CO_2 excess persists.

The electrolyte balance of the serum for the arterial blood of boys 10 to 17 years of age, is given in Table II and is compared there with similar data obtained from the results of a study of 12 normal adult males by Dill, Edwards and Consolazio (19). The results indicate that there is a gradual rise in the serum bicarbonate concentration as the child passes through adolescence. This rise in bicarbonate is not compensated by a corresponding reduction in chloride, proteinate, phosphate or lactate ions, nor by an increase in the sodium ion. We have found no evidence in the literature to suggest a compensating increase in the other basic ions, potassium, calcium and magnesium, during the transition from childhood to adulthood. The compensation for the rise in serum bicarbonate which occurs as the age of the child increases would, therefore, seem to lie in a reduction in concentration of one or more as yet undetermined anions, probably organic in nature.

DISCUSSION

The present report is one of the few studies of hemoglobin levels in children in which hemoglobin concentration in the blood has been measured by determination of the oxygen capacity of the blood by the Van Slyke method. Previous studies include those of Mugrage and Andresen (20, 21) for Denver children, which are believed to show higher values than those for the United States as a whole because of a compensatory reaction to high altitude, and that of Robinson (1) which includes data for about 25 children of the Boston area. Most other studies have used colorimetric or photometric methods which, for lack of a pure hemoglobin solution, usually have been calibrated against solutions whose hemoglobin concentrations have been measured by oxygen capacity or blood iron determinations. A mass study of the population in Hessen, Germany (22), employed the copper sulfate specific gravity method which was standardized against Van Slyke oxygen capacity determinations.

A summary of various hemoglobin studies that have been made in the United States, Canada, Great Britain, Germany and Australia (1, 20–37) is given in Figure 3. It is evident that there is little agreement concerning the normal hemoglobin level of a child of a given age and the changes with growth. In general it is recognized that after a low level, reached during the first half year or year of life, the hemoglobin level rises at rates depending upon the age of the child, and reaches the adult level at an age which as yet is not too clearly defined. It is now known that much of the confusion which exists is due both to technical errors and to the great complexity of factors which effect the hemoglobin level. This question has been well discussed in Chapter II of the report of the British Committee on Hemoglobin Surveys (26).

For children up to 12 years, and for the adult group, the hemoglobin levels of our study [4] approximate the higher hemoglobin levels reported in the literature for the various ages. On the other hand, between 13 and 17 years our values are lower than most of those of other studies, especially at 14, 16 and 17 years. Variation in the resting period prior to drawing the blood sample in the three groups of subjects may in part explain this discrepancy. Most of the subjects from 11 to 17 years of age had remained in the recumbent position for two hours or more prior to the drawing of the arterial blood sample, for measurements of lung capacity and of blood and available fluid volumes, while the younger children and the young adults had been in the recumbent position fifteen to thirty minutes when the sample was drawn. In the case of the younger children, furthermore, a complete resting state could not be maintained in the period preceding and during the taking of the sample. The effect of rest and posture on the plasma volume is well known. Recently Widdowson and McCance (39) showed that after two hours rest in bed the mean hemoglobin concentration decreased from 14.1 to 13.4 gm. per 100 ml. of blood.

[4] Hemoglobin concentrations, in grams per 100 ml. blood, were calculated by dividing HbO_2 capacity values in vol. % by 1.34.

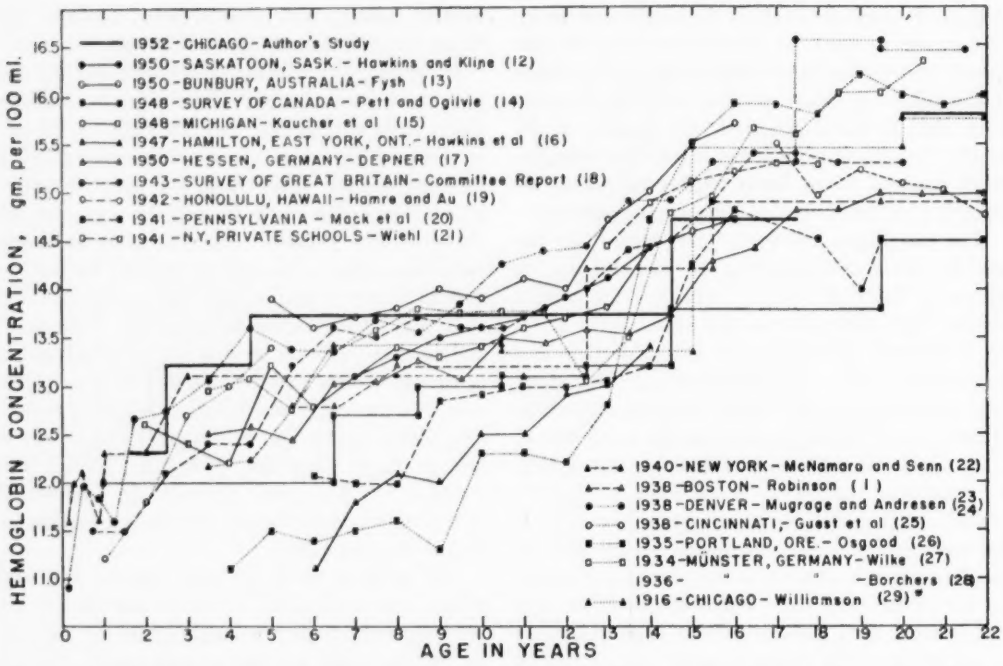
1952 - CHICAGO - Author's Study
1950 - SASKATOON, SASK. - Hawkins and Kline (12)
1950 - BUNBURY, AUSTRALIA - Fysh (13)
1948 - SURVEY OF CANADA - Pett and Ogilvie (14)
1948 - MICHIGAN - Kaucher et al (15)
1947 - HAMILTON, EAST YORK, ONT. - Hawkins et al (16)
1950 - HESSEN, GERMANY - DEPNER (17)
1943 - SURVEY OF GREAT BRITAIN - Committee Report (18)
1942 - HONOLULU, HAWAII - Hamre and Au (19)
1941 - PENNSYLVANIA - Mack et al (20)
1941 - N.Y. PRIVATE SCHOOLS - Wiehl (21)
1940 - NEW YORK - McNamara and Senn (22)
1938 - BOSTON - Robinson (1)
1938 - DENVER - Mugrage and Andresen (23) (24)
1938 - CINCINNATI - Guest et al (25)
1935 - PORTLAND, ORE. - Osgood (26)
1934 - MÜNSTER, GERMANY - Wilke (27)
1936 - " " - Borchers (28)
1916 - CHICAGO - Williamson (29)
HEMOGLOBIN CONCENTRATION, gm. per 100 ml.
AGE IN YEARS

FIG. 3. RELATION OF AGE TO THE HEMOGLOBIN CONCENTRATION OF THE BLOOD IN CHILDREN AND YOUNG MALE ADULTS —A COMPARISON OF THE AUTHOR'S RESULTS WITH THOSE OF OTHER STUDIES IN THE UNITED STATES, GREAT BRITAIN, CANADA, AUSTRALIA, AND GERMANY

With three exceptions the values shown on the chart include data for children and young adults of the male sex. These exceptions are 1) the author's study which includes data for children of both sexes under 11 years, 2) the study of McNamara and Senn (30) which gives data for infants and children of both sexes through 11 years, and 3) that of Guest, Brown and Wing (33) which gives data for both sexes from infancy through 5 years.

Williamson's original values are generally believed to be too high, due probably to the use of impure hemoglobin as the standard for a spectrophotometric method. Following the example of Peters and Van Slyke (38), the original mean values have been reduced by multiplying each by 0.92.

Since oxygen capacity was determined by the gasometric method without correction for the errors which Roughton, Darling and Root (7) showed may be introduced by unequal drainage of cells and plasma from the walls of the tonometer and by the presence of inactive hemoglobin, the mean value of 95.9 per cent for the arterial saturation of normal adults is approximately 2 per cent lower than the recent results of Drabkin and Schmidt (40), Comroe and Walker (41), and Wood (42). Earlier determinations (1, 43–49) of oxygen saturation by the gasometric tonometer technic gave mean values for adults which on the whole agree well with that of this study. Values found by Gibbs, Lennox, Nims and Gibbs (45) and by Cullen and Cook (46) tend to be lower.

Our results for children tend to be higher than values reported by Preston and Ordway (50) who found a mean value of 93.9 per cent for 14 children 7 to 14 years of age by the Roughton-Scholander microgasometric method (51). They agree better with the mean value of 95.3 per cent which Brinkman and Jonxis (52) found for 20 normal children by means of a photoelectric method. These authors found a trend toward slightly lower values, with a mean of 93.6 per cent, in infants between 2 and 7 months of age. Robinson (1) found that arterial saturation varied little with age between 10 and 40 years of age, although after 40 years it tended to decrease slightly. Our results agree with those of Robinson for children over 6 years and for young adults. Robinson did not include data for younger children.

We have noted a trend toward slightly lower values of arterial oxygen saturation in the very young child. Although this difference is small, consideration of its origin has theoretical interest. Factors which might cause a small difference in arterial saturation involve differences in alveolar oxygen pressure, alveolar-arterial pO_2 gradients, and the position or shape of the oxygen dissociation curve.

While the lower arterial saturation of the adult of advanced years may be attributed to an increase in the alveolar-arterial oxygen tension gradient with increasing age (1, 53), the assumption of a correspondingly high gradient in the young child does not seem probable. Causes for a high gradient, such as venous admixture from anastomoses in the coronary or pulmonary circulation, areas in the lungs that are perfused but not well ventilated, or a high diffusion gradient for oxygen across the alveolar membrane, are unlikely to appear in the young child to a greater degree than in the older child or the adult.

We have shown in an earlier report (54) that the position of the oxygen dissociation curve is displaced to the right in the child as compared to the adult. The average age of the children studied was under 6 years. If it could be shown that the dissociation curve lies farther to the right in the very young child and gradually moves to the left as the child grows, this would offer a simple explanation for the small difference in arterial saturation which was found. Proof for such a shift in the position of the dissociation curve would be difficult because of the small differences involved and the comparatively large individual variation. A study of a limited number of older children gave no evidence of a return to the adult position.

Under basal conditions the rate of pulmonary ventilation in the young child is higher than it is in the older child. According to Robinson's investigations (1) this is due both to a higher respiratory rate and to a tidal air volume which is high in relation to body size and vital capacity. Using the Haldane end-expiratory method for collecting samples of alveolar air, Robinson found a mean alveolar pO_2 of 105 mm. in children 7 to 12 years of age as compared with a mean value of 98 mm. in children of 13 to 15 years. Corresponding values for younger children were not measured, but Robinson noted a higher ventilatory rate in the child of 5 and 6 years as compared with that of the 8 to 12 year old child. Presumably this would lead to a greater rate of alveolar ventilation, a higher alveolar pO_2, with subsequently a slightly higher arterial oxygen saturation rather than the lower value found in the present study. It is quite possible that the apparent contradiction lies in the fact that the basal state did not truly exist because crying and a certain amount of struggling could not be avoided in younger children.

The effects of crying cannot be predicted since crying involves varying degrees both of breathholding and of hyperventilation. One tends to compensate the other. In the case of children of 2 and 3 years the acid-base balance of the arterial blood in Figure 3 shows practically no evidence of CO_2 excess, characteristic of breathholding, nor of CO_2 deficit, characteristic of overventilation. Since crying did occur, it must be presumed that the effects of breathholding have balanced those of hyperventilation, at least so far as the CO_2 equilibrium is concerned. However, oxygen is known to diffuse through the alveolar membrane more slowly than CO_2, hence it cannot be said with certainty that the breathholding phase of crying has not lowered the arterial saturation by lowering the alveolar oxygen tension.

The effects upon the arterial saturation of the young child due to the physical effort of struggling should also be considered. Evidence in the literature concerning the effect of exercise on arterial saturation is conflicting, but all authors agree that any effect in a normal, healthy adult will be small unless the exercise is severe and fatiguing. Himwich and Barr (55) have shown that the arterial saturation of healthy adults rises slightly during moderate exercise but may decrease if the exercise is severe enough. Riley, Lilienthal, Proemmel, and Franke (56) found arterial saturations of 91.7, 92.5, 95.2 and 93.2 during exercise on the treadmill or bicycle ergometer as compared with three values during rest of 95.2, 95.3 and 98.8. Later, Riley and his collaborators reported similar results with three more normal adults (57). Experiments of Montgomery and his associates (58), in which normal persons 9 to 60 years of age walked on a treadmill at 1.7 M.P.H., showed negligible changes in arterial saturation as measured by the ear oximeter; values ranged from -2.0 to 0 percentage points, with a mean value of 0.3 percentage points. Our own experience,

using the ear oximeter to determine arterial saturation in healthy children and adults during moderate exercise on the treadmill, leads us to the conclusion that while arterial saturation may decrease by one or two percentage points during the first minute or so of exercise it rises to the resting level or above this when adequate pulmonary and circulatory adaptations are made to meet the increased demand for oxygen. It is possible that these demands were not met at the time that the arterial sample was drawn in the young child.

The triaxial charts of Figure 3 show clearly the acid-base relationships in arterial blood at the different ages of childhood. The good agreement of our data for adults with those of Dill, Edwards and Consolazio (19), which were secured by the same methods, gives confidence that the findings for children are not due to technical errors but represent actual deviations from the adult state. The heavy lines of the chart follow those of Shock and Hastings (18). They represent the pathway of change from the normal, due to respiratory factors which cause CO_2 excess or deficit, and to metabolic factors which result in acid or alkali excess or deficit.

By reference to the guide lines it can be seen that the low arterial CO_2 content which was found in most of the children of 2 and 3 years is due to metabolic acidosis. In only two cases is there evidence of hyperventilation. Metabolic acidosis may be due to addition of fixed acid to the blood or loss of base from the blood. Experimental data are lacking which could determine which factor is responsible for the acid-base pattern which was observed in the very young child. However, since Kelsey and Leinbach (59) have shown by the conductivity method of Sunderman (60) that mean total base concentrations are higher in children of 2 to 6 years than in older children, excess of acid in the blood appears to be the more probable factor.

It is of interest to consider the possible composition of the acids which may have replaced bicarbonate in the blood serum of the child of 2 to 6 years. We can only conjecture, since anions other than bicarbonate were not determined in children of this age. Of the possible anions it would seem true that only chloride and organic acids could differ from the adult pattern in quantities great enough to balance the decrease in bicarbonate which was observed.

The arterial samples were drawn before the child had breakfast, approximately 16 hours after the meal of the preceding evening. Young children are known to be particularly sensitive to ketogenesis since acetone bodies appear in the urine and breath after comparatively short periods of fasting. It may be possible that the decreased bicarbonate concentration is caused by an increase in ketone acids in the blood. Gamble, Ross, and Tisdall (61) studied changes in acid-base equilibrium during the prolonged periods of fasting which were used as a therapeutic measure in epileptic children and found a marked decrease in both bicarbonate and chloride ions after three or four days of fasting, due to the presence of ketone acids. In experiments of Shaw and Moriarty (62), which followed the changes in alkaline reserve in epileptic children on consecutive days of fasting, the decrease in alkaline reserve did not appear until the second or third day of the fast, although breath acetone was stronger the first day than on those following. On the other hand, Wilson, Levine and Rivkin (63), noted a marked decrease in the CO_2 content of the blood of normal children 6 to 12 years of age after 24 to 30 hours of fasting. It has not yet been proven whether or not the tendency to ketosis is marked enough in children of 2 and 3 years to reduce the bicarbonate concentration by an average of 3 mEq. per liter after a fast of no more than 17 hours.

Our results agree with those of Robinson (1) in showing an increase in the alkaline reserve or T_{40} value of the blood with increasing age from early childhood to the adult state. Leenhardt and Chaptal (64) found a similar trend in a study in which the alkaline reserve of the plasma was determined from early infancy to 14 years. Robinson suggested that the lower value in early childhood may represent a compensation to loss of CO_2 by overventilation, since his study had shown that young children have very high rates of lung ventilation both in rest and during exercise. A reduced alkaline reserve is known to occur in cases of chronic hyperventilation (65, 66) and is also found in the blood of residents at high altitudes (49, 67, 68). As has been shown, such an explanation is contradicted in the case of the very young children by the position of all but two of their values in the acid-base diagram. As the child grows, the acid-base pattern is shifted in the

direction of respiratory alkalosis to positions which represent the combined effect of mild hyperventilation and acidosis. The net effect may be considered either a compensated metabolic acidosis or a compensated respiratory alkalosis, depending on which is the primary factor. It would seem reasonable to consider the change with growth as a gradual change in the metabolic processes in the sense that the production of organic acids is reduced and compensation is achieved by the high respiratory rate. A study of the complete electrolyte balance in this age range might test the accuracy of this assumption.

If the rapid respiratory rate of the child under 12 years is the primary factor in the production of a low alkaline reserve, analogy with a state of chronic hyperventilation caused either by disease or by residence at high altitudes suggests that compensation would be achieved chiefly by a rise in serum chloride concentration. Although data concerning the serum chloride concentrations of normal children of all ages are not abundant in the literature, available information gives no evidence that the chloride concentration of children of any age is different from that of the adult. A number of investigators (69–74) have shown that the chloride concentration of the blood serum of the newborn infant tends to be higher than that of the adult, and Marples and Lippard (70) found that a high serum chloride in the newborn balanced a low alkaline reserve, but Overman, Etteldorf, Bass, and Horn (73) observed that after the first day or two of life the plasma chloride concentration decreased and the mean level of 104 mEq. per liter in infants of 11 to 26 months was identical with that found by Erickson, Williams, Hummel, and Macy (74) in children 5 to 9 years of age. Robinson (1) found the plasma chloride concentration in arterial blood remarkably constant throughout the entire age range from 8 to 80 years, the average varying only from 104.0 to 105.3 mEq. per liter in the various groups.

It is evident that while available data agree that the alkaline reserve of the blood tends to be low in the very young child, and that the adult level is not reached until the age of twelve years or later, a satisfactory explanation of the electrolyte balance must await further experimental work.

SUMMARY

The blood gases, oxygen saturation and acid-base balance have been determined in arterial blood samples from 41 children of both sexes 2 to 10 years of age, from 63 boys 11 to 17 years of age and from 9 young male adults. All were physically normal and in good health at the time the samples were taken.

From the plotted data certain trends with age were observed; these were analyzed statistically. The results may be summarized as follows:

1. Hemoglobin concentration of the blood, as measured by the oxygen capacity, increases with increasing age from a level of 12.3 G. per 100 ml. at 2 years to a level of 14.7 G. per 100 ml. at 15 to 17 years. The mean value for young male adults was found to be 15.8 G. per 100 ml.

2. A mean percentage arterial oxygen saturation of 94.2 per cent for children of 2 to 4 years is significantly lower than that of 95.8 per cent for children of 7 to 17 years and of 95.9 per cent for young adults.

3. The CO_2 content of arterial blood is found to be relatively low in children of 2 and 3 years, with a mean level of 40.1 vol. per cent. In children above 3 years of age the mean level rises gradually from 44.4 vol. per cent at 4 to 6 years to 48.5 vol. per cent in the adult. The alkaline reserve of the blood and plasma shows a similar trend.

4. Arterial CO_2 tension increases at 13 years from a previous level of 38 mm. Hg to the adult level of 41 mm. Hg.

5. The mean pH_a of arterial blood varies between 7.38 and 7.40 for all age groups except the youngest. A mean pH_a of 7.35 was found in children of 2 and 3 years.

6. In children of 2 and 3 years displacement of the acid-base balance from the adult pattern is shown to be almost entirely in the direction of fixed acid excess or metabolic acidosis. Between 4 and 11 years the acid-base balance appears to be the resultant of slight acid excess and CO_2 deficit. In boys of 12 years and over, evidence of acid excess almost disappears and the acid-base pattern is shifted rather toward slight CO_2 excess.

7. A study of the electrolyte pattern of the serum in the arterial blood of boys 10 to 17 years of age has shown that, although the bicarbonate concentration rises slightly with increasing age,

this rise is not balanced by a compensatory reduction in the concentration of chloride, proteinate, phosphate or lactate ions of the serum, nor by a corresponding rise in serum sodium.

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ARTERIAL BLOOD GASES AND ACID-BASE BALANCE IN CYANOTIC CONGENITAL HEART DISEASE [1]

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The purpose of this report is to present data concerning the arterial blood gases and the acid-base balance in patients with cyanosis due to congenital heart disease. Since the study is related to cyanosis and abnormalities of the blood consequent to this, no distinction is made between the various anatomical defects producing cyanosis.

Compensatory or adaptive mechanisms in the presence of cyanosis related to congenital heart disease have been the subject of a number of studies (1–6). The present study was undertaken to obtain further information concerning the arterial blood gases in this disease, especially in relation to age and in respect to the influence of arterial unsaturation on the acid-base balance.

Sixty cyanotic patients were studied, varying in age from 1 to 36 years. The methods used have been described in a preceding report (7).

RESULTS

Because age has been found to affect the concentrations of the blood gases (7), especially the HbO_2 capacity, the CO_2 content and the alkaline reserve, it was necessary to evaluate the results for an individual of a given age in terms of deviations from the mean values for that age. Normal values for children of different ages are given in the preceding report (7). Table I gives the results of single tests on each of 60 patients with cyanotic congenital heart disease, in terms both of absolute values and of deviations from the normal. In Figures 1 and 2 deviations from the normal are plotted for each age group against a background which represents the range of variation found in normal, healthy individuals of the same age group. Table II gives the results of repeated tests on seven individuals. It includes cases of pulmonary stenosis in which pulmonary blood flow was increased by aortic-pulmonary artery anastomosis or pulmonary valvulotomy.

Certain differences in distribution with respect to age are evident in the data of Table I and Figures 1 and 2. Arterial oxygen saturation below 50 per cent was found only in children of 7 years and younger. The fact that all but one of the adults had arterial saturations above 79 per cent is misleading and an artifact of sampling. Of the adult group only one was believed to have pulmonary stenosis. It should be noted that in the reports of Talbott et al. (1), Bing, Vandam, and Gray (8), and Suarez, Chiodi, Fasciolo, and Taquini (3), of 33 patients with cyanotic congenital heart disease who were over 18 years of age, 15 were found to have arterial saturations below 75 per cent. However, only one of these patients had a saturation below 60 per cent. The great majority of patients of our study who had HbO_2 capacities within the normal range were younger children. On the other hand, the majority of very high HbO_2 capacities, over 32 vol. per cent, were found in older children and adults. One child of 6 years was found to have a HbO_2 capacity of 32.5 vol. per cent. In regard to the alkaline reserve of the plasma there seems to be no special trend with age. The lowest values were found in children of 7 and 8 years. It may be significant that in spite of the fairly high arterial oxygen saturations found in the adults of this study, all values for alkaline reserve were below the normal range of variation. In the case of the arterial pCO_2, about a third of the children of 12 years and younger had values above the normal mean, whereas in children above that age and in adults all values but one lay below the normal mean. The great majority of the lower than normal pH_a values were found in children between 6 and 10 years.

Since the data of Table I are arranged in the order of increasing arterial saturation, it can be

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TABLE I

Oxygen saturation, HbO_2 capacity, plasma CO_2 T_{40}, pCO_2 and pH_a of arterial blood of patients with cyanotic congenital heart disease*

No.	Subject	Age	Sex	Arterial saturation	HbO_2 capacity		Plasma CO_2 T_{40}*		Arterial pCO_2		Arterial pH_a	
					Value found	Dev. from normal	Value found	Dev. from normal	Value found	Dev. from normal	Value found	Dev. from normal
		yr.		*per cent*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*mm.*	*mm.*		
1	S. D.	2	F	21.0	14.5	− 2.0	37.3	−11.1	41.7	+ 4.4	7.227	−.122
2	R. E.	2	M	21.4	28.6	+12.1	37.4	−11.0	40.2	+ 2.9	7.266	−.083
3	P. S.	1	F	21.6	18.0	+ 1.5	39.6	− 8.8	42.1	+ 4.8	7.257	−.092
4	R. B.	6	M	40.5	28.6	+10.2	35.7	−18.9	37.8	− 0.2	7.244	−.155
5	J. M.	7	M	40.6	31.1	+12.7	34.6	−20.0	35.9	− 2.1	7.244	−.155
6	J. A.	2	M	41.7	25.6	+ 9.1	37.3	−11.1	35.0	− 2.3	7.279	−.070
7	H. R.	2	M	46.9	14.0	− 2.5	42.6	− 5.8	25.5	−11.8	7.423	+.074
8	J. G.	4	F	49.8	30.9	+13.2	41.5	−12.0	39.3	+ 2.0	7.297	−.093
9	M. P.	14	M	52.0	33.8	+15.4	43.3	−12.2	40.8	− 0.5	7.308	−.073
10	J. W.	9	M	58.7	32.8	+14.4	33.6	−21.0	38.3	+ 0.3	7.203	−.201
11	S. X.	15	M	59.1	35.1	+16.7	41.6	−13.9	31.6	− 9.7	7.348	−.033
12	V. S.	7	F	61.8	26.6	+ 8.2	45.3	− 9.3	37.9	− 0.1	7.334	−.065
13	W. B.	6	M	63.2	28.4	+10.0	40.8	−12.7	32.9	− 4.4	7.322	−.077
14	M. M.	36	F	63.4	35.1	+14.6	45.6	−12.6	30.2	−10.9	7.399	−.003
15	L. C.	14	F	65.4	32.4	+14.0	41.2	−14.3	32.8	− 8.5	7.328	−.053
16	C. F.	6	M	65.6	32.5	+14.1	42.4	−11.1	41.6	+ 4.3	7.283	−.107
17	J. R.	10	F	65.7	25.0	+ 6.6	50.6	− 4.0	39.5	+ 1.5	7.369	−.034
18	V. J.	3	F	68.3	19.7	+ 2.0	43.6	− 4.8	40.7	− 3.4	7.289	−.060
19	N. H.	4	M	68.6	29.9	+11.5	46.4	− 7.1	29.4	− 7.9	7.407	+.017
20	A. F.	5	M	70.4	27.8	+ 9.4	49.4	− 4.1	34.5	− 2.8	7.392	+.002
21	B. G.	7	M	70.6	27.8	+ 9.4	45.9	− 8.7	37.8	− 0.2	7.333	−.066
22	L. S.	17	M	71.2	26.4	+ 6.7	49.5	− 6.3	29.7	−11.6	7.434	+.053
23	J. S.	10	M	72.1	27.3	+ 8.9	49.0	− 5.6	33.4	− 4.6	7.398	−.006
24	B. M.	13	M	72.2	22.7	+ 4.3	54.2	− 1.3	27.5	−13.8	7.502	+.121
25	C. L.	2	M	73.0	16.6	− 0.1	49.1	+ 0.7	32.4	− 4.9	7.407	+.058
26	P. M.	5	F	73.3	21.0	+ 2.6	45.9	− 7.6	29.4	− 7.9	7.401	+.011
27	S. D.	10	M	74.2	31.2	+12.8	46.2	− 8.4	38.4	+ 0.4	7.333	−.071
28	R. B.	8	M	74.3	28.1	+ 9.7	48.9	− 5.7	39.4	+ 1.4	7.351	−.053
29	R. J.	2	M	74.9	19.1	+ 2.6	43.6	− 4.8	34.7	− 2.6	7.330	−.019
30	P. K.	5	F	75.4	22.7	+ 4.3	45.5	− 8.0	37.7	+ 0.4	7.328	−.062
31	R. F.	4	M	76.8	24.6	+ 6.9	42.5	−11.0	35.2	− 2.1	7.313	−.077
32	R. D.	10	M	77.0	36.5	+18.1	43.8	−10.8	27.7	−10.3	7.391	+.013
33	R. M.	6	M	77.4	17.4	− 1.0	49.6	− 3.9	44.7	+ 7.4	7.311	−.092
34	L. Z.	24	F	79.0	22.7	+ 2.2	55.5	− 2.9	28.5	−12.6	7.496	+.094
35	E. V.	11	M	79.3	25.3	+ 6.9	46.1	− 9.4	34.4	− 3.6	7.355	−.049
36	G. H.	35	F	79.4	29.1	+ 8.6	48.0	−10.2	46.8	+ 5.7	7.296	−.106
37	R. G.	25	M	79.7	25.7	+ 5.2	52.2	− 6.0	34.3	− 6.8	7.408	+.006
38	G. N.	31	M	79.9	22.6†	+ 2.1	50.9	− 7.3	38.8	− 2.3	7.367	−.035
39	C. L.	4	M	80.0	18.2	+ 0.5	41.1	−12.4	23.5	−13.8	7.412	+.022
40	D. R.	8	M	80.7	24.6	+ 6.2	48.6	− 6.9	34.5	− 6.8	7.379	−.002
41	S. L.	7	F	80.8	23.9	+ 5.5	43.7	−10.9	33.1	− 4.9	7.340	−.001
42	M. C.	16	F	81.0	26.2	+ 7.0	45.6	− 9.9	34.4	− 6.7	7.351	−.030
43	D. B.	19	M	82.4	35.7	+15.2	47.6	−10.6	37.2	− 3.9	7.352	−.029
44	D. M.	8	M	82.8	22.5	+ 4.1	49.3	− 5.3	29.5	− 8.5	7.429	+.025
45	J. T.	5	F	83.6	23.8	+ 5.4	49.2	− 4.3	34.7	− 2.6	7.377	−.022
46	I. B.	23	F	83.8	25.2	+ 4.7	51.5	− 6.7	33.7	− 7.4	7.407	+.005
47	A. G.	12	M	84.9	22.5	+ 4.1	47.6	− 7.0	30.0	− 8.0	7.407	+.003
48	C. K.	9	M	85.9	21.6	+ 3.2	50.9	− 3.7	35.9	− 2.1	7.387	−.017
49	M. M.	10	F	87.1	20.0	+ 1.6	46.6	− 8.1	42.2	+ 4.2	7.298	−.105
50	J. M.	12	M	88.0	21.6	+ 3.2	55.6	+ 1.0	26.5	−11.5	7.519	+.116
51	B. Z.	31	M	89.0	28.5	+ 8.0	51.6	− 6.6	37.1	− 4.0	7.380	−.022
52	M. D.	12	F	89.8	17.2	− 1.2	52.7	− 2.8	34.0	− 4.0	7.421	+.022
53	P. T.	25	M	89.9	24.4	+ 3.9	54.3	− 3.9	37.9	− 3.2	7.399	−.003
54	B. N.	16	M	90.1	27.9	+ 8.2	50.0	− 5.5	33.5	− 7.8	7.394	+.013
55	P. B.	9	F	90.5	18.4	0.0	48.7	− 5.9	40.2	+ 2.2	7.330	−.074
56	W. G.	9	M	90.8	18.4	0.0	52.3	− 2.4	45.7	+ 7.7	7.323	−.080
57	B. V.	10	M	91.7	21.1	+ 2.7	53.0	− 1.6	32.8	− 5.2	7.428	+.025
58	P. C.	4	F	91.7	17.2	− 0.5	53.0	− 0.5	43.7	+ 6.4	7.340	−.050
59	R. W.	4	M	91.9	18.6	+ 0.2	47.1	− 6.4	39.7	+ 2.4	7.317	−.073
60	T. A.	9	M	92.7	26.1	+ 7.7	48.6	+ 0.2	28.7	− 8.6	7.420	+.016

* Plasma CO_2 T_{40}, or alkaline reserve, is defined as the CO_2 content of plasma from fully oxygenated blood which has been equilibrated with CO_2 at a tension of 40 mm. Hg.

† The moderately low HbO_2 capacity in this case is due to repeated phlebotomy.

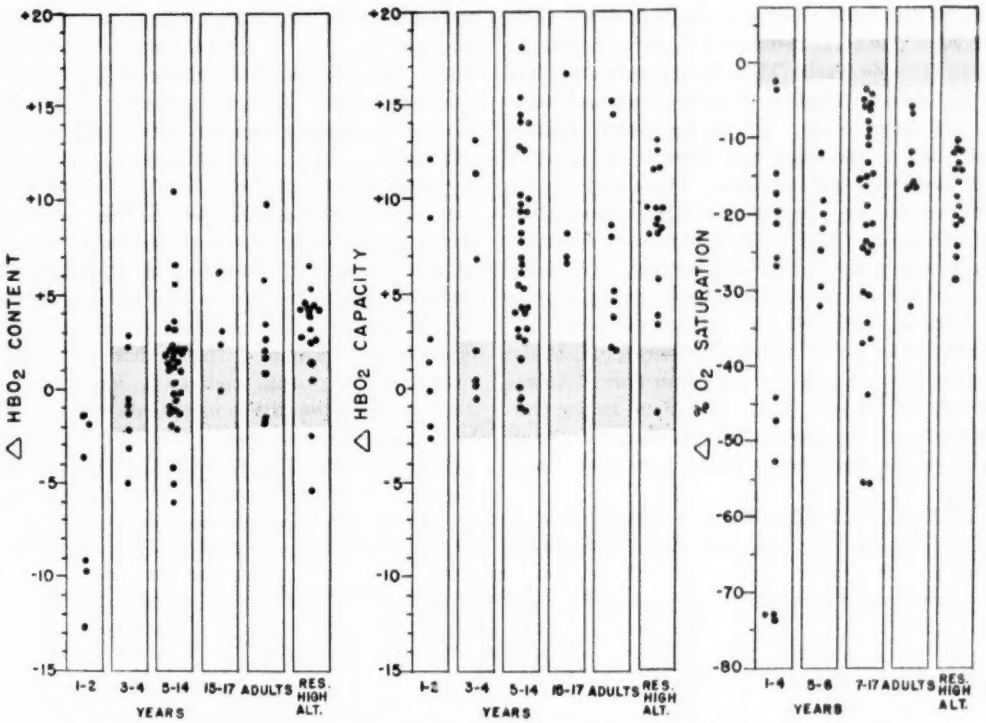

FIG. 1. ARTERIAL HbO_2 CONTENT, HbO_2 CAPACITY, AND PERCENTAGE OXYGEN SATURATION OF THE BLOOD OF PATIENTS WITH CYANOTIC CONGENITAL HEART DISEASE, SHOWN AS DISTRIBUTIONS OF THE DEVIATIONS FROM THE NORMAL MEAN FOR THE VARIOUS AGE GROUPS

seen that rough relationships exist between the degree of arterial unsaturation and the extent of the deviations of the HbO_2 capacity, alkaline reserve and arterial pH_a from the normal mean. These relationships are more clearly apparent in Table III which gives mean values for the three groups into which the material was divided on the basis of arterial unsaturation. The more marked deviations from the normal tend to occur in patients with arterial saturations below 70 per cent. In each group there are marked exceptions.

The acid-base balance of the arterial blood of patients with cyanotic congenital heart disease is shown in Figure 3, in which the individual data for $(HCO_3)_a$, pCO_2 and pH_a are plotted on triaxial charts according to the method of Hastings and Steinhaus (9) and of Shock and Hastings (10). The shaded area in each chart represents the range of variation which we have found for normal healthy children of corresponding age groups and for adults (7). It is evident that in the great majority of cases the acid-base balance may be described as that due to fixed acid excess or to a combination of fixed acid excess and CO_2 deficit.

Data concerning serum chloride, protein and lactic acid were obtained for 15 of the patients with cyanotic congenital heart disease in the hopes of observing changes in the electrolyte balance of the serum which might compensate for the reduced bicarbonate concentration. The data include two independent determinations on three of the patients, in one case separated in time by three years. The results in Table IV show that in half of the cases the sum of the three ions which constitute the great part of the anion moiety of the serum electrolytes, bicarbonate, chloride, and proteinate, falls within the normal range of variation, while in the other half the sum of the three major anions is below normal. The table shows

clearly that the difference in the sum of the three anions is due almost entirely to a difference in the mean chloride level. Thus, in approximately half of the cases a reduction in the bicarbonate level is almost balanced by a rise in the serum chloride level, but in the other half there is no apparent rise in chloride concentration. Those in the group without chloride compensation tend to have lower saturations and higher HbO_2 capacities, although the values of the two groups overlap. The mean pH_a of the group without chloride compensation is 7.332 as compared with a mean value of 7.382 for the other group, but the low mean pH_a of the former group is due to lower than normal values in only half of the group. The blood lactate concentration of the group without chloride compensation is not significantly higher than in the other group.

In Figures 1 and 2 the last column of each group contains corresponding data for residents at high altitude, taken from the reports of Dill et al. (11–13) and of Hurtado and Aste-Salazar (14). The triangles and squares of Figure 3 also show these data. They are given for the purpose of comparison since both residents at high altitude and patients with cyanotic congenital heart disease represent hypoxic states to which the individual has become acclimatized over a long period of time. Both groups show approximately the same degree of arterial unsaturation, although the spread of values in cyanotic congenital heart disease is greater and much lower values are sometimes found. Figure 2 shows that the alkaline reserve of the plasma tends to be lower in residents at high altitude than in patients with cyanotic congenital heart disease. Table III includes data for residents at high altitude in terms of mean deviations from the normal. Of the three groups of patients with cyanotic congenital heart disease which are shown in this table, the second group,

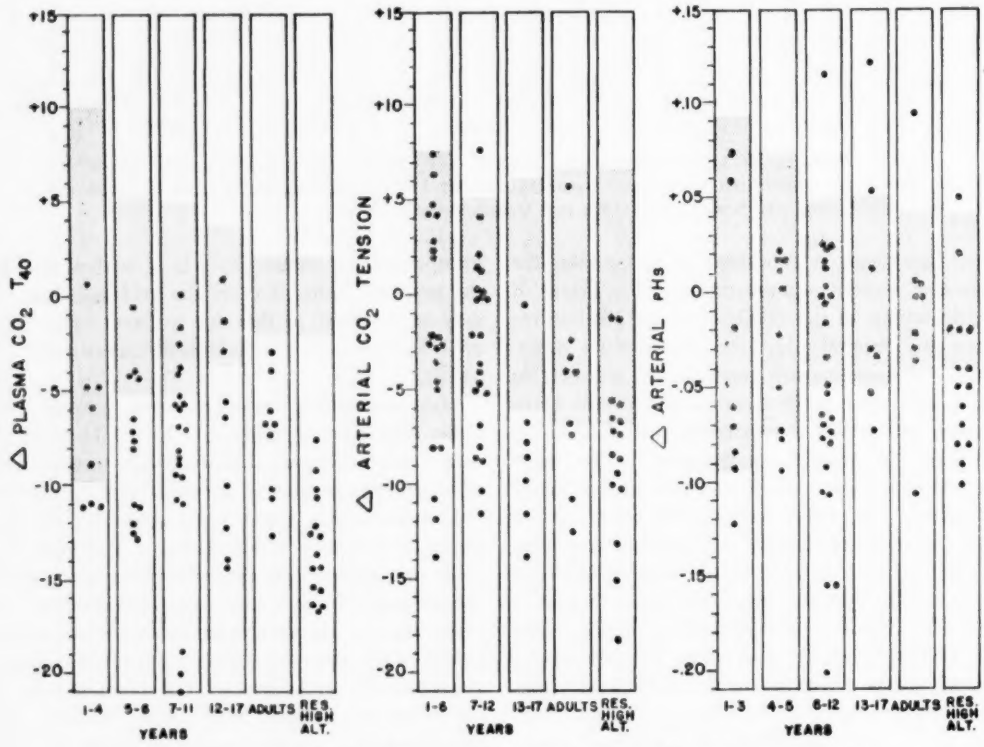

FIG. 2. PLASMA CO_2 T_{40}, ARTERIAL pCO_2, AND ARTERIAL pH_a OF PATIENTS WITH CYANOTIC CONGENITAL HEART DISEASE, SHOWN AS DISTRIBUTIONS OF THE DEVIATIONS FROM THE NORMAL MEAN FOR THE VARIOUS AGE GROUPS

TABLE II

Results of repeated tests on the same individual

No.	Date	Subject	Age		Notes	Arterial saturation	HbO_2 capacity	Plasma* CO_2 T_{40}	Arterial pCO_2	Arterial pH_a
			Yr.	*Mo.*		*per cent*	*vol. %*	*vol. %*	*mm. Hg*	
5	2–25–42	J. Mu.	2	7		54.9	27.1	41.7	43.4	7.266
	9– 8–43		4	2		54.2	31.4	40.0	47.7	7.232
	12– 3–46		7	4		40.6	31.1	34.6	35.9	7.244
	4–22–47		7	9		53.1	27.9	33.5	40.8	7.186
	Post-op., 5–14–47		7	10	Aortic-pulmonary artery anastomosis, 4–24–47	72.2	23.9	47.0	44.9	7.295
	3–10–49		9	8		75.2	25.7	53.0	34.7	7.422
17	10–14–47	J. R.	9	8	Blalock anastomosis, 3–29–47	71.8	18.7	54.6	47.3	7.340
	Post-op., 11–18–47		9	9	Obliteration of anastomosis, 10–17–47	65.7	25.0	50.6	39.5	7.369
22	4–11–52	L. S.	16	10		71.2	26.4	49.5	29.7	7.434
	Post-op., 10–15–52		17	4	Pulmonary valvulotomy, 4–14–52	96.6	19.7	57.0	48.6	7.387
27	6–12–44	S. D.	8	0		68.8	31.5	47.1	34.7	7.373
	10–11–46		10	4		70.8	31.1	42.7	36.3	7.314
	10–17–46		10	4		74.2	31.1	46.2	38.4	7.333
	10–28–46		10	5	Phlebotomy, 10–24–46	62.6	27.4	49.3	39.0	7.365
	12–12–46		10	6	Iron medication begun 10–30–46	66.1	29.4	42.8	35.2	7.325
	9– 9–47		11	3		77.1	27.9	47.0	33.9	7.372
	Post-op., 10– 8–47		11	4	Aortic-pulmonary artery anastomosis, 9–14–47	84.3	24.0	52.4	33.5	7.422
	12– 8–47		11	6		83.8	25.4	51.4	31.4	7.431
	3– 8–49		12	9		83.2	26.0	53.4	32.4	7.438
28	5– 5–47	R. B.	8	0		74.3	28.1	48.9	39.4	7.351
	Post-op., 5–23–47		8	1	Aortic-pulmonary artery anastomosis, 5–6–47	77.6	21.9	52.3	33.3	7.426
	8–20–52		13	2		74.1	24.2	47.6	30.2	7.401
29	1–10–49	R. J.	2	0		74.9	19.1	43.6	34.7	7.330
	Post-op., 2– 4–49		2	1	Aortic-pulmonary artery anastomosis, 1–11–49	76.8	16.4	47.5	31.1	7.403
50	12–11–47	J. Mr.	10	10		84.0	22.0	51.7	30.1	7.447
	1–18–49		11	11		88.0	21.6	55.6	26.5	7.519
	4– 2–51		14	1		81.0	23.1	54.9	37.1	7.415
	Post-op., 6–29–51		14	4	Pulmonary valvulotomy, 6–13–51	95.1	20.9	53.6	39.7	7.375

* Plasma CO_2 T_{40}, or alkaline reserve, is defined as the CO_2 content of plasma from oxygenated blood which has been equilibrated with CO_2 at a tension of 40 mm. Hg.

with arterial saturations of 70 to 88 per cent, compares well in arterial saturation with residents at high altitude. It is evident that for comparable degrees of arterial unsaturation the blood of residents at high altitudes has a slightly higher HbO_2 capacity, a much lower alkaline reserve and arterial pCO_2, and a slightly lower pH_a. The acid-base picture in Figure 3 is quite similar in both groups, *i.e.*, a combination of fixed acid excess and CO_2 deficit.

The post-operative results in Table II give information as to the effects of increased pulmonary blood flow on the arterial blood gases and the acid-base balance. In four cases of Tetralogy of Fallot, two, J. Mu. and S. D., showed marked improvement in arterial saturation, but little change was observed in R. B. and R. J. Not shown in the table is the fact that R. J. showed marked clinical improvement and little evidence of cyanosis when seen four years later. L. S. and J. Mr., who had diagnoses of pulmonary stenosis and atrial septal defect, showed arterial saturations of 96.6 and 95.0 per cent respectively following valvulotomy. A reduction of HbO_2 capacity occurred in all cases. Those patients who had a marked rise in arterial saturation also showed a marked rise in the alkaline reserve of the plasma. In the cases of J. Mu. and S. D. the alkaline re-

TABLE III

*Relation of the degree of arterial unsaturation to the deviations from the normal of the HbO_2 capacity and the acid base balance of the blood of patients with cyanotic congenital heart disease—a comparison of these deviations from the normal with comparable values in native residents at high altitude**

No. of cases	Arterial saturation per cent	Mean deviations from the normal mean			
		HbO_2 capacity vol. %	Plasma CO_2 T_{40}† vol. %	Arterial pCO_2 mm.	Arterial pH_a
		Patients with cyanotic congenital heart disease			
10	93–88	+ 2.9 ± 1.21‡	− 3.5 ± 0.79	−1.4 ± 1.81	−0.023 ± 0.0136
31	88–70	+ 5.8 ± 1.31	− 5.4 ± 1.18	−4.6 ± 1.29	−0.010 ± 0.0104
12	70–40	+12.9 ± 0.72	−13.8 ± 1.72	−3.1 ± 1.44	−0.083 ± 0.0185
		Residents at high altitudes			
15	85–68	+ 8.5 ± 0.95	−13.2 ± 0.73	−8.4 ± 1.19	−0.043 ± 0.0106

* Data for residents at high altitude are taken from the reports of Dill et al. (11–13) and of Hurtada and Aste-Salazar (14).

† Plasma CO_2 T_{40}, or alkaline reserve, is defined as the CO_2 content of plasma from fully oxygenated blood which has been equilibrated with CO_2 at a tension of 40 mm. Hg.

‡ The figures following the ± signs represent the standard error of the mean.

serve of the plasma rose to levels within the normal range in spite of the fact that their arterial blood still contained a considerable degree of venous admixture. Their pH_a levels, which previously had been consistently low, rose to normal levels after an aortic-pulmonary artery anastomosis had been made. In the case of J. R. the preoperative values are comparable to the postoperative values in the other patients. A Blalock anastomosis had been performed some time before but cardiac decompensation followed. Subsequent surgical obliteration of the anastomosis reduced the arterial saturation, raised the HbO_2 capacity, and decreased the alkaline reserve of the plasma. It also reduced the arterial pCO_2 which presumably had been high because of pulmonary congestion.

DISCUSSION

The fact that the great majority of patients with cyanotic congenital heart disease have a normal or higher than normal arterial oxygen content is due to the fact that the great majority have a high HbO_2 capacity. The high arterial oxygen content loses much of its value in the presence of reduced arterial saturation since the arterial oxygen tension rather than its concentration determines the level of the mean capillary oxygen pressure and the resultant pressure gradient or diffusion pressure head between capillaries and tissues. Studies of cyanotic congenital heart disease by Bing et al. (2) and Ernsting and Shephard (6) have emphasized the great part played by the sigmoid shape of the oxygen dissociation curve in reducing the arterial-mean capillary oxygen tension gradient, in contrast to the small effect of the greatly increased HbO_2 capacity. The latter exerts its effect on the mean capillary oxygen pressure by raising the venous level of saturation since, of two bloods equally saturated, the one with high HbO_2 capacity will remain more highly saturated after equal quantities of oxygen per unit of blood have been transferred to the tissues.

It is of interest to find that 12 patients with cyanotic congenital heart disease, or 20 per cent of the number studied, did not have an elevated HbO_2 capacity. Half of these had mild cyanosis and arterial saturations between 87 and 92 per cent. The majority of the remainder were infants not over 2 years of age. The fact that blood counts in these infants showed elevated values, with red cell counts of 6.2 to 8.5 million per cu. mm., suggests that the low HbO_2 capacity is due to a nutritional deficiency. Administration of ferrous sulfate to one infant raised his HbO_2 capacity. Talbott and associates' (1) summary of the literature shows several cases of congenital heart disease in which the blood HbO_2 capacity was not elevated. Burchell, Taylor, Knutson, and Wood (4) have also noted a case with 68 per cent saturation and with an HbO_2 capacity of only 15.2 vol. per cent.

The arterial saturation found in patients with cyanotic congenital heart disease covers a wide

range. Eight cases showed arterial saturations below 50 per cent. It is probably significant that saturations below 25 per cent were found only in infants and that no saturation below 50 per cent was observed in children over 7 years of age. A very low arterial saturation suggests a poor prognosis unless remedial operative procedures can be used. Operation was attempted in the case of two infants with arterial saturations below 25 per cent but death followed. The third infant with this low saturation survived only a few months after she was studied. Autopsy revealed transposition of the great vessels. There is suggestive evidence that correction of the iron deficiency present in cyanotic infants with low HbO_2 capacity improves their status.

We believe that many of the saturation values found in arterial samples in children may represent the lower limit of a variable quantity which is determined by factors which regulate both the amount of right to left shunt and the venous oxygen level. Saturation levels determined by the ear oximeter are usually higher because they are measured under conditions that are less disturbing to the patient than are arterial punctures.

The finding of a low alkaline reserve of the plasma in patients with cyanotic congenital heart disease has been reported previously by Talbott et al. (1) and by Suarez, Chiodi, Fasciolo, and Taquini (3). It has also been suggested by the low CO_2 contents of arterial blood found in Talbott's summary of the literature (1), and in the serum of arterial blood as reported by Bing et al. (2).

Talbott et al. (1) first described the analogy between the tissue anoxia of patients with cyanotic congenital heart disease and that of persons living under the low oxygen pressure of high altitudes. He attributed the low alkaline reserve of the blood in these patients to the effects of chronic hyperven-

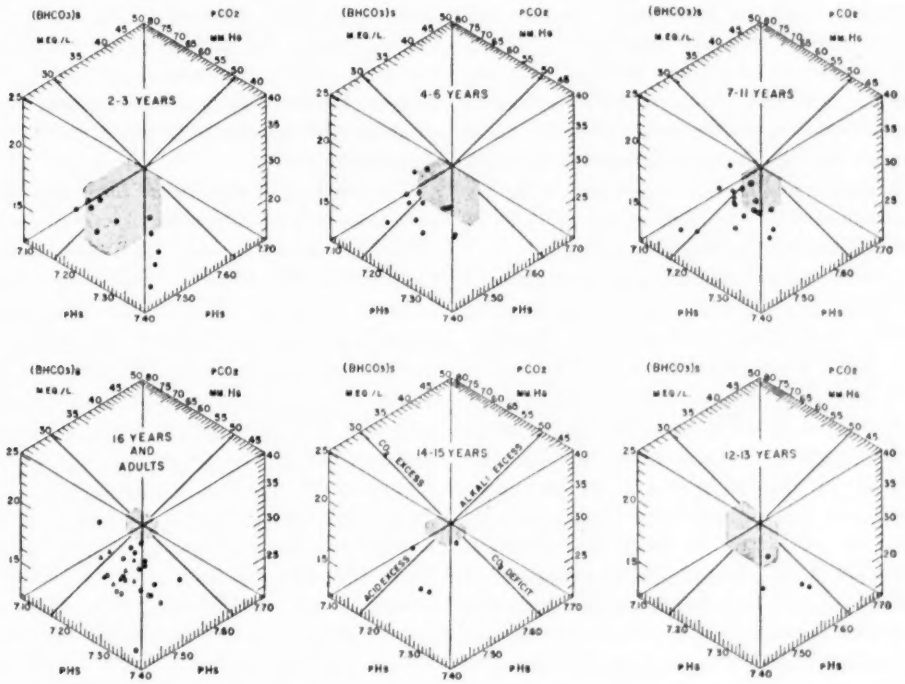

FIG. 3. ARTERIAL $(HCO_3)_s$, pCO_2 AND pH_s OF THE BLOOD OF PATIENTS WITH CYANOTIC CONGENITAL HEART DISEASE, PLOTTED ON TRIAXIAL COORDINATES

The shaded areas represent the range of normal variation for the various age groups as determined by ±2 standard deviations from the mean value of each group.

tilation, but he also recognized that some other factors caused the acidosis which was observed in the patient he had studied so extensively and in several other cases reported in the literature. Bing et al. (2) found that the minute volume of respiration was increased above the normal in 29 out of 30 cases of cyanotic congenital heart disease, but the arterial pCO_2 and the alkaline reserve were reduced proportionately and the arterial pH_a remained within normal limits. Suarez, Chiodi, Fasciolo, and Taquini (3) found hyperventilation to be characteristic of the six patients with this disease which they studied and arterial pH_a in the normal range or slightly low.

Bing et al. (2) emphasized the difference in conditions under which tissue anoxia is produced in patients with congenital heart disease and in residents at high altitudes. In the latter a low alveolar oxygen pressure produces the low arterial pO_2 and saturation. Hyperventilation is beneficial in that it raises the alveolar pO_2. In patients with cyanotic congenital heart disease on the other hand low arterial pO_2 and saturation levels are caused by venous admixture through abnormal channels. Beneficial effects of hyperventilation are questionable in such patients since the blood that passes through the lungs is exposed to a high oxygen tension and is well oxygenated, while the blood shunted from the right to the left side of the heart does not pass through the lungs.

Presumably hyperventilation rises in response to an anoxic stimulus both in patients with cyanotic congenital heart disease and in residents at high altitude. Dill, Talbott and Consolazio (13) showed that an essential process in acclimatization to high altitude consists first of hyperventilation to increase alveolar oxygen pressure, followed later by a reduction of the alkaline reserve of the plasma by kidney action to the point where the normal ratio between the pCO_2 and the serum bicarbonate is reached. Both Dill, Talbott and Consolazio (13) and Hurtado and Aste-Salazar (14) found that the arterial blood serum of permanent residents at high altitude is characterized by a lowering of the pCO_2 proportional to that of the alkaline reserve, with a normal or slightly low pH_a, in contrast to the elevated pH_a of the arterial blood of temporary residents at high altitude. Patients with cyanotic congenital heart disease resemble residents at high altitude in that they are acclimatized to their state of anoxia. Our results show that for a given degree of arterial unsaturation the blood of patients with cyanotic congenital heart disease shows less of a depression of alkaline reserve than that of residents at high altitude. Since for equal degrees of alveolar hyperventilation the arterial pCO_2 must remain higher in the blood of patients with cyanotic congenital heart disease because of the venous admixture it contains, there is need for less decrease in the alkaline reserve of the blood in these patients in order to maintain a normal arterial pH_a.

The case of cyanotic congenital heart disease studied by Talbott et al. (1) was unusual because of the low arterial pH_a which was observed. The present report shows 32 per cent of pH_a values below the lower limit of normal. The great majority of pH_a values below 7.30 were found in samples showing less than 60 per cent saturation and are therefore associated with large venous-arterial shunts. In such cases of severe hypoxia a low pH_a offers compensation by raising arterial oxygen tension, hence mean capillary oxygen pressure, through the Bohr effect on the oxygen dissociation curve.

The low pH_a of the arterial blood and the lack of balance in the common electrolytes of the serum in cases of severe hypoxia led Talbott to suggest the presence of increased concentrations of organic acids in the blood. Since tissue metabolism apparently proceeds at relatively low levels of oxygen tension, it is conceivable that enzyme reactions involved in muscle metabolism operate at a more anaerobic level, with production of lactic acid in larger quantities than normal. Our results agree with those of Hallock (15), Bing et al. (2), and Havel and Watkins (5) in showing that in the resting state the lactic acid concentration in the blood of cyanotic patients with congenital heart disease is not significantly higher than in the normal person, and that the slightly elevated lactic acid levels that are occasionally found are not great enough to explain the deficiencies in the electrolyte balance.

It is important to note also that although Bing et al. (2) found that the basal oxygen consump-

TABLE IV

Serum electrolyte concentrations in the arterial blood of patients with cyanotic congenital heart disease

Age *yr.*	Subject	Oxygen saturation *per cent*	HbO_2 capacity *vol. %*	$(HCO_3)_a$ *mEq./L.*	$(Cl)_a$ *mEq./L.*	$(Proteinate)_a$ *mEq./L.*	$(HCO_3)_a + (Cl)_a + (Proteinate)_a$ *mEq./L.*	$(Lactate)_a$ *mEq./L.*	$(Sodium)_a$ *mEq./L.*	pH_a
				Group I. Normal values for $(HCO_3)_a + (Cl)_a + (Proteinate)_a$						
3	W. B.	63.4	23.2	18.8	110.0	16.6	145.4	1.9		7.414
6	J. T.	83.6	23.8	20.0	106.3	18.1	144.4	2.9		7.377
7	V. S.	61.8	26.6	19.8	105.6	19.6	145.0	1.2		7.234
10	J. S.	72.1	27.3	20.3	105.1	20.4	145.8	1.0		7.398
16	B. N.	90.1	27.9	20.2	106.3	18.0	144.5	1.8		7.394
16	M. C.	81.0	26.2	18.7	106.6	17.5	142.8	1.0		7.351
Adult	R. G.	79.7	25.7	21.2	108.2	15.6	145.0	0.6		7.408
Mean		76.0	25.8	19.9	106.9	18.0	144.7	1.5		7.382
				Group II. Low values for $(HCO_3)_a + (Cl)_a + (Proteinate)_a$						
3	N. H.	84.1	26.0	17.6	99.8	17.8	135.2	1.5		7.432
4	J. M.	54.3	31.4	19.7	100.6	15.0	135.3	1.2	133.6	7.232
5	A. F.	70.4	27.8	20.7	103.2	14.0	137.9	1.6		7.392
6	R. B.	40.5	28.6	16.1	104.2	18.0	138.3	2.9		7.244
6	R. B.	39.2	26.5	16.9	103.2	17.8	137.9	2.6		7.300
7	J. M.	40.6	31.1	15.3	104.2	16.6	136.1	1.7	146.3	7.244
7	W. G.	70.6	27.8	19.8	101.7	18.5	140.0	1.6		7.333
8	D. M.	82.8	22.5	19.2	104.1	17.3	140.6	1.8		7.429
10	S. D.	66.1	29.4	18.1	98.3	19.5	135.9	2.1	139.8	7.325
11	S. D.	77.1	35.7	19.3	101.5	16.4	137.2	1.7		7.372
Adult	D. B.	82.4	35.7	20.2	103.3	16.3	139.8	1.1		7.352
Mean		64.4	29.3	18.4	102.2	17.0	137.7	1.8		7.332

tion was usually below the normal mean in these patients, that finding has not been corroborated in our laboratory or by other investigators such as Burchell, Taylor, Knutson, and Wood (4), Suarez, Chiodi, Fasciolo, and Taquini (3) and Ernsting and Shephard (6). We must conclude that metabolic requirements for the resting state are met by adequate oxygen consumption in patients with marked arterial unsaturation. At present it is not known whether this is made possible by modifications in the complex of enzymatic processes involved in tissue metabolism and whether the production of organic acids other than lactic acid are increased under these conditions. The problem requires further study. The possibility of alterations in renal excretion also must be studied in this connection.

SUMMARY

The blood gases and acid-base balance of the blood were determined in arterial samples from 60 patients with cyanotic congenital heart disease who varied in age from 1 to 36 years. The results may be summarized as follows:

Arterial saturation varied from 21 to 93 per cent. Saturations below 50 per cent were found only in children under 8 years of age.

In 80 per cent of the cases HbO_2 capacities were more than 2 vol. per cent higher than the normal mean for the appropriate age. Of those with normal or below normal HbO_2 capacities, half were cases with arterial saturation between 87 and 92 per cent, and the majority of the remainder were infants in whom a nutritional iron deficiency was suspected. Very high HbO_2 capacities, over 32 vol. per cent, were usually found only in older children and in adults.

By means of high HbO_2 capacities the oxygen content of the arterial blood was maintained within the normal range in all but 15 per cent of the cases.

The alkaline reserve of the plasma tended to be reduced, for approximately two-thirds of the observed values were below the lower limit of normal. Arterial pCO_2 was below the normal range in only one-third of the cases. As a result, values for arterial pH_a were below the lower limit of normal in approximately one-third of the cases.

A rough relationship was found to exist be-

tween the degree of arterial unsaturation and the extent of the deviations of the HbO_2 capacity, alkaline reserve and arterial pH_a from the normal mean.

Deviations of the acid-base characteristics of the serum from the normal were found to be due in most cases either to fixed acid excess or to a combination of fixed acid excess and CO_2 deficit.

Calculation of a partial electrolyte balance showed that in some cases the reduced bicarbonate concentration of the serum was balanced by a rise in serum chloride concentration. In other cases there was no evidence of replacement of serum bicarbonate by chloride, nor by increased lactate concentration. The question of a corresponding reduction of total fixed base or an increase in other organic acids has not been investigated sufficiently.

The determination of arterial blood gases in samples drawn post-operatively, after pulmonary blood flow had been increased by aortic-pulmonary artery anastomosis or pulmonary valvulotomy, showed a marked rise in the alkaline reserve and the arterial pH_a of the blood, in addition to the well-known increase in arterial saturation and reduction of HbO_2 capacity.

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THE FAILURE OF INTRACRANIAL PRESSURE-VOLUME CHANGE TO INFLUENCE RENAL FUNCTION [1]

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A variety of lesions of the central nervous system may be associated with disturbances of renal function characterized by either the increased urinary loss of sodium and chloride (1–3) or the retention of salt (3–8). It has also been postulated that changes in the excretion of salt, secondary to alterations in the volume of blood or extracellular fluid, are mediated by a "volume receptor" located in the cranial cavity (9–12). The following experiments were therefore undertaken in order to investigate the influence of alterations in intracranial venous volume and pressure, and cerebrospinal fluid (CSF) volume and pressure on renal function.

METHODS

Female dogs were anesthetized with sodium pentobarbital (30 mg. per Kg.) except for two experiments in which chloralose (75 mg. per Kg.) was used. Urine was collected through an indwelling catheter. The bladder was washed out with distilled water after each collection and emptied completely by blowing out with air. Glomerular filtration rate was measured by the clearance of exogenous creatinine (C_{Cr}) (13) and renal plasma flow was estimated from the clearance of paraminohippurate (C_{PAH}) (14). Urine was analyzed for sodium with a flame photometer and for chloride titrimetrically (15). Cisternal, femoral arterial, and jugular venous pressures were measured with Statham strain gauges and recorded on a Brush oscillograph. Pressure in the jugular vein was obtained by passing a polyethylene catheter up to the level of the intracranial jugular bulb.

EXPERIMENTAL PROCEDURE AND RESULTS

Four groups of experiments were performed. Saline, 0.85 per cent, was administered intravenously at 4 or 5 cc. per min. by means of a constant infusion pump. After two hours, urinary flows of about 3 cc. per min. were established. Three ten minute "Control" periods were then obtained, followed by three ten minute "Experimental" periods, and by three ten minute "Recovery" periods. Urine was discarded for five minutes immediately before and after the thirty minute "Experimental" period.

(1) *Control:* Seven dogs were studied during nine ten minute clearance periods in order to ascertain the normal variations in urinary flow, clearances of creatinine and PAH, and excretion of sodium and chloride, which might occur during this period of time.

(2) *Neck compression:* Eight dogs were subjected to acute compression of the neck with a blood pressure cuff at 60 mm. Hg during the thirty minute "Experimental" period. This procedure produced a threefold elevation in pressure in the jugular bulb and a secondary twofold rise in cerebrospinal fluid pressure, without altering arterial pressure.

(3) *Neck compression and cerebrospinal fluid drainage:* Seven dogs were subjected to acute compression of the neck with a blood pressure cuff at 60 mm. Hg with simultaneous drainage of about 15 cc. to 20 cc. of CSF to maintain the CSF pressure at minus 150 mm. water during the thirty minute "Experimental" period. This procedure was designed to produce an increase in both volume and pressure of intracranial veins, while decreasing the volume and pressure of cerebrospinal fluid. The pressure in the jugular bulb was tripled but arterial pressure was unaltered.

(4) *Intracisternal injection of saline:* Twenty dogs were subjected to an increase in CSF pressure by the introduction of normal saline into the cisterna magna, at the rate of 0.5 cc. per min. during the thirty minute "Experimental" period, sufficient to maintain a CSF pressure of 500 mm.

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TABLE I

The mean value for each thirty minute period is stated. The means of the difference between each value for the control and experimental periods, and for the experimental and recovery periods, were tested for significance using Fisher's t test for the significance of the mean of a unique sample (16). The value of P in each case is stated, and cannot be regarded as significant if greater than 0.05.

	Control (7 Exps.)			Neck compression (8 Exps.)			Neck compression + CSF drainage (7 Exps.)			Cisternal injection (20 Exps.)		
	Control	Control	Control	Control	Experimental	Recovery	Control	Experimental	Recovery	Control	Experimental	Recovery
Urine flow *cc./min.*	3.4	4.3	3.9	3.2	3.4	3.6	3.9	4.1	4.0	4.1	3.9	4.0
p>		0.1	0.9		0.4	0.3		0.5	0.3		0.3	0.7
UV Na *u eq./min.*	445	493	442	312	311	340	376	373	380	309	306	328
p>		0.5	0.1		0.9	0.2		0.9	0.7		0.9	0.2
UV Cl *u eq./min.*	432	446	421	289	280	324	392	398	401	321	330	351
p>		0.8	0.2		0.7	0.1		0.9	0.8		0.8	0.3
C_{PAH} *cc./min.*	201	191	169	219	199	194	258	242	225	229	201	183
p>		0.05	0.01		0.01	0.01		0.01	0.01		0.01	0.01
C_{Cr} *cc./min.*	60	63	60	66	66	68	75	76	76	65	63	61
p>		0.2	0.6		0.9	0.8		0.5	0.5		0.1	0.1

water. Neither venous pressure in the jugular bulb nor arterial pressure was altered.

Urinary flow, the excretion of sodium and chloride, and the clearances of creatinine and PAH were not significantly altered, when compared with the control studies, by any of the experimental procedures described above. The data are detailed in Table I. It should be noted that the C_{PAH} gradually fell during the entire period of infusion in all experiments. This change may be attributed to the influence of pentobarbital anesthesia (17).

In view of the known effects of pentobarbital anesthesia on renal function (17), two additional experiments were done using chloralose anesthesia. In these, cisternal injection of saline was also without effect on the excretion of salt.

To ascertain whether the saline load in these experiments might interfere with the urinary response to the experimental procedure, three experiments were performed on dogs who received no intravenous infusion. In two dogs, the excretion of sodium and chloride, during a one hour period during which the CSF pressure was maintained at 500 mm. water by cisternal injection of saline, did not differ significantly from the salt excretion during the one hour control period or the one hour recovery period. In one dog similarly studied, but subjected to a one hour period of neck compression and CSF drainage, the excretion of sodium and chloride was not altered. Thus the pattern of salt excretion was not influenced by these procedures in the presence or absence of a saline load.

DISCUSSION

Acute alterations in the volume of plasma or extracellular fluid have been reported to induce alterations in the urinary excretion of sodium and chloride (18, 19). However, there is no evidence that the disturbances in electrolyte excretion associated with lesions of the central nervous system are related to alterations in the intracranial volume of blood or extracellular fluid. Harrison and coworkers have reported that compression of the neck of the sitting man results in an increased urinary excretion of sodium and chloride (9, 10). This procedure had little or no effect in recumbent subjects. One explanation for this difference might relate to differences in the volume of the intracranial veins rather than to the pressure of this system. Since, in the sitting position, the intracranial CSF pressure is lower than it is in the recumbent posture, jugular compression in the sitting position might be expected to produce greater distention of intracranial veins than if the

subject were supine. There are no methods for directly measuring the volume of CSF or the intracranial venous volume. However, these two volumes vary inversely if the total volume of the cranial cavity is kept essentially constant (20). Thus cerebrospinal fluid drainage was used in the experiments of Group 3 to induce distention of the intracranial veins. The absence of any change in salt excretion despite compression of the neck (Group 2) and reduction of the CSF pressure to minus 150 mm. water (Group 3), suggests that an acute increase in intracranial venous volume is without effect on renal function. In the experiments of Group 4, a primary increase in the pressure and the volume of the CSF was produced, presumably associated with a decrease in the intracranial venous volume (20). Under these circumstances no alteration in renal function was noted. In four of the twenty experiments performed, the excretion of sodium and chloride fell to 30 to 50 per cent of the control value when the CSF pressure was elevated, and immediately returned to the control rate of excretion when the pressure was reduced. This change was not statistically significant ($p > 0.8$).

When the CSF pressure is elevated to the level of the arterial diastolic pressure, there follows a secondary rise in arterial pressure (21). This is associated with an acute reduction in urinary flow, a manifestation of extreme renal vasoconstriction secondary to presumed anoxic stimulation of the vasomotor center (22). In the present experiments, however, the CSF pressure was elevated to a level well below diastolic arterial blood pressure and alterations in systemic blood pressure or renal plasma flow did not occur.

Thus, neither an increase nor a decrease in intracranial venous distention or cerebrospinal fluid pressure and volume could be demonstrated to affect renal hemodynamics, urinary flow, or excretion of sodium and chloride. It is also of interest that an acute reduction of cerebral blood flow, produced by ligation of the carotid arteries bilaterally in the dog, is similarly without effect on the excretion of sodium and chloride (23).

These experiments fail to support the hypothesis that there is an intracranial "volume receptor" influencing the renal excretion of sodium and chloride in the anesthetized dog.

SUMMARY

Alterations in intracranial venous pressure and venous volume, and the pressure and volume of cerebrospinal fluid, have been studied for their effect on renal function in the anesthetized dog. The excretion of sodium and chloride, urinary flow, and clearances of creatinine and paraminohippurate are not significantly influenced by altering pressure-volume relationships of the cerebral venous system and the cerebrospinal fluid.

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EFFECT OF SURGICAL SYMPATHECTOMY ON THE SENSITIVITY TO EPINEPHRINE OF THE BLOOD VESSELS OF MUSCULAR SEGMENTS OF THE LIMBS [1, 2]

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Increased sensitivity to epinephrine of blood vessels deprived of their sympathetic nerve supply was first demonstrated in man by Freeman, Smithwick, and White (2). In patients with Raynaud's disease they found that the fall in temperature in the fingers resulting from daily intravenous epinephrine infusions became greater on the eighth to eighteenth day after the limbs had been sympathectomized. The increase in sensitivity was thought to be much greater following postganglionic denervation than after preganglionic decentralization (3). In monkeys, Ascroft (4) estimated that the postganglionic operation caused more than three times as much hypersensitivity as the preganglionic. By contrast Fatherree, Adson, and Allen (5) considered that both operations caused about the same degree of hypersensitivity, which opinion is supported by Duff (6). All these studies were confined to the blood vessels of the skin.

Concerning the effect of sympathectomy on the sensitivity of the blood vessels in skeletal muscle in man, information is incomplete. In a study of the circulatory differences between hands and forearms it was incidentally noted by Grant and Pearson (7) that the dilator response of the forearm to epinephrine was increased in one patient following sympathectomy. In patients with peripheral arterial disease sensitization of the muscle vessels of the lower limb has been reported to follow preganglionic section (8).

In their investigation of the blood flow in human skeletal muscle Allen, Barcroft, and Edholm (9) showed that, during intravenous epinephrine infusions, marked vasodilatation of the forearm occurred early, both in normal and in sympathectomized limbs. Pursuing this study of the effect of epinephrine on muscle blood flow, Duff and Swan (10) reported that during intravenous epinephrine infusions the initial marked dilatation was succeeded by a second phase of moderate dilatation in normal but not in sympathectomized limbs. Because of its absence in chronically sympathectomized limbs this secondary vasodilatation was at that time thought to be an indirect vasomotor effect mediated by the sympathetic nerves. Re-examination of their data in the light of some subsequent critical experiments now reveals that the difference which they found between normal and sympathectomized limbs may be ascribed largely to vascular hypersensitivity in the latter.

In the present paper these additional data are reported, and are incorporated with those of Duff and Swan (10); the whole material being interpreted to provide evidence that hypersensitivity of the vessels of skeletal muscle in the upper and lower limbs may result from pre- and postganglionic sympathectomy in man.

SUBJECTS

In 15 experiments the blood flow responses to epinephrine in the forearm and calf were measured plethysmographically in five healthy and three sympathectomized subjects additional to the 30 healthy and 13 sympathectomized cases studied by Duff and Swan (10). In the combined group of patients sympathectomy had been performed for Raynaud's disease, hyperhidrosis or causalgia, and one subject had suffered traumatic sympathectomy of one upper limb. The ages of these subjects ranged from 19 to 44 years and none had any evidence of impaired circulation through the muscle of the extremities studied. Tests had been performed to make certain that each sympathectomized limb had complete and effective interruption of the appropriate sympathetic pathway. Failure

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[2] Presented at the Twenty-Fifth Annual Meeting of the Central Society for Clinical Research, Chicago, Illinois, November 7, 1952 (1).

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to exhibit vasodilatation or sweating following indirect heating sufficient to raise the body temperature by 1° C. was taken as good evidence of the absence of vasomotor nerves.

METHODS

The venous occlusion plethysmograph, which was employed, is based on the principle that brief application of a pressure which occludes the veins but not the arteries of a limb causes congestive swelling of the tissues distally, swelling at a rate exactly equal to that of the arterial inflow (11). The limb to be tested was inserted into a sheath of thin rubber of appropriate size, to either end of which had been sealed stout rubber diaphragms with holes neatly to admit the limb. The limb was adjusted so that the most muscular segment of forearm or calf was enclosed in the rubber sheath. The latter was then placed inside a rigid plastic plethysmograph with the diaphragms in close apposition to its apertures, to the rims of which they were now sealed by screws and gaskets. A segment of forearm or of calf thus lay within the plethysmograph, enclosed in a watertight thin rubber sheath. Inflatable rubber cuffs were applied to the limbs proximal and distal to the plethysmograph, following the general technique of Barcroft and Edholm (12, 13). The plethysmographs were filled with water maintained at 34 ± 1° C. (14), care being taken to ensure thorough mixture of the water to obtain a uniform temperature.

The circulation distal to the plethysmograph was excluded, during periods of observation, by inflating the distal cuff at a pressure exceeding the arterial blood pressure. When the proximal cuff was inflated for five to ten seconds at a pressure of 30 to 60 mm. Hg, the consequent swelling of the tissue within the plethysmograph was transmitted to a volume recorder, which was inscribing on a revolving drum. Allowing, between measurements, an interval of at least twice the duration of the venous occlusion, it was possible to obtain records each half minute. From these the forearm or calf blood flow rate was calculated and expressed as ml. blood flow per minute, per 100 ml. tissue volume as determined by water displacement.

Throughout each experiment, saline was infused continuously into the basilic vein by means of an electrically driven infusion apparatus delivering 4 ml. per minute at a constant rate. After a control period of 20 minutes the plain saline was replaced for exactly 10 minutes with saline containing the requisite amount of synthetic levo-epinephrine bitartrate (supplied by British Drug Houses, Ltd.) after which the plain saline infusion was resumed. Records of blood flow and of blood pressure were obtained regularly each half minute throughout both the saline and the epinephrine periods. For all the intravenous studies the amount of epinephrine delivered was 10 μg. per min. The characteristic symptoms and changes in respiration, heart rate and blood pressure have already been described (10).

In each test the magnitude of the response during each phase was separately assessed. The initial vasodilatation, measured by the highest single value recorded by the plethysmograph during the first phase, was related to the level of flow immediately before the start of the epinephrine infusion. During the second phase the average flow during the last four minutes of the infusion was compared with the average during the last four minutes of the control (pre-epinephrine) period. Since a substantial number of observations relating to this second phase have already been published (10) the further results of the present study have not been tabulated, but the combined data have been subjected to statistical analysis and presented in Tables II and III.

RESULTS

1. *Comparison of normally innervated and sympathectomized forearms.* The typical findings in a patient with unilateral cervicodorsal sympathectomy, caused by traumatic avulsion of the brachial plexus, are illustrated in Figure 1.

With the first infusion of epinephrine the initial rise in flow on the sympathectomized side reached 15.4 ml. per 100 ml. per min., or 12.3 ml. above the control flow, as compared with a peak of 13.4 ml., or 11.3 ml. above control, on the normal side. During the second phase the contrast was more marked. On the normal side the blood flow returned to a level about double the resting rate, but in the sympathectomized forearm the flow returned to, or slightly below, the control level.

In addition to these differences the second epinephrine infusion revealed that the vasodilatation started some one-half minute earlier in the sympathectomized than in the normal forearm. This accelerated response was observed on several occasions in sympathectomized limbs.

2. *Comparison of normally innervated and sympathectomized calves.* The blood flow in both calves of a young woman who had undergone unilateral lumbar preganglionic sympathectomy one month previously for causalgia of the right foot is charted in Figure 2. On the sympathectomized side the initial vasodilatation occurred one-half minute earlier and reached a peak of 11.8 ml., or 9.3 ml. above control, as compared with a peak of only 6.8 ml. or 4.1 ml. above control in the normal limb. During the subsequent phase irregular vasodilatation was present in the normal but not in the sympathectomized calf.

3. *Responses of groups of limbs during initial phase.* Observations from 29 individuals including 23 of the subjects originally studied by Duff

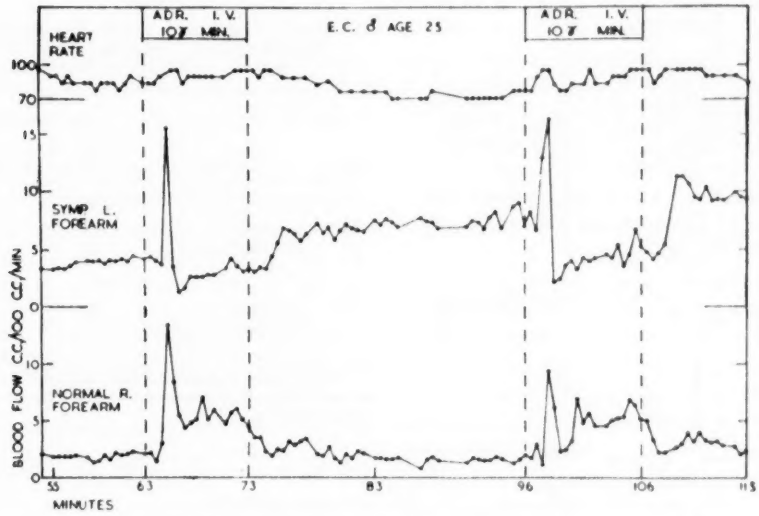

FIG. 1. BLOOD FLOW IN BOTH FOREARMS OF A PATIENT WITH A SYMPATHECTOMIZED LEFT UPPER LIMB

The greater initial vasodilatation and subsequent vasoconstriction on the sympathectomized side during intravenous infusions of epinephrine at 10 μg. per min. are shown.

TABLE I

Maximum rises in blood flow in normal and sympathectomized forearms and calves during first phase of intravenous epinephrine infusions at 10 μg. per min.

Normal			Sympathectomized		
Blood flow ml./100 ml./min.					
Control	Peak	Rise above control	Control	Peak	Rise above control
		Forearms			
2.0	9.3	7.3	1.7	7.7	6.0
2.0	10.5	8.5	1.9	13.0	11.1
2.1	7.7	5.6	2.4	13.3	10.9
2.1	7.3	5.2	2.6	10.5	7.9
2.1	6.7	4.6	3.1	15.4	12.3
2.1	13.4	11.3	3.2	12.0	8.8
2.4	9.0	6.6	4.2	11.4	7.2
2.7	7.2	4.5	4.2	15.6	11.4
4.0	13.0	9.0	4.5	9.2	4.7
4.3	9.5	5.2	5.2	13.0	7.8
Ave. 2.6	9.4	6.8	Ave. 3.3	12.1	8.8
		Calves			
2.1	6.1	4.0	2.2	9.3	7.1
2.3	6.2	3.9	2.3	11.6	9.3
2.3	5.3	3.0	2.5	11.8	9.3
2.7	6.8	4.1	2.6	13.2	10.6
3.4	7.8	4.4	3.1	6.2	3.1
4.2	10.8	6.6	4.6	12.1	7.5
4.5	8.1	3.6	5.4	15.4	10.0
5.2	7.6	2.4			
5.5	11.6	6.1			
5.6	11.4	5.8			
Ave. 3.8	8.2	4.4	Ave. 3.2	11.4	8.1

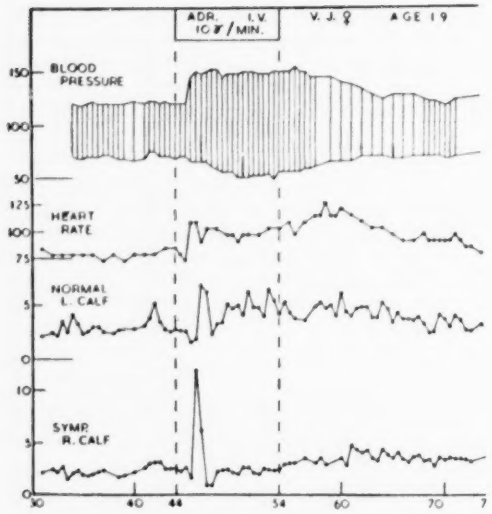

FIG. 2. ARTERIAL BLOOD PRESSURE, HEART RATE, AND BLOOD FLOW IN BOTH CALVES OF A PATIENT WHOSE RIGHT LOWER LIMB WAS SYMPATHECTOMIZED ONE MONTH PREVIOUSLY

With the intravenous infusion of epinephrine, the initial vasodilatation is seen to start earlier and reach a higher peak on the sympathectomized side. Some dilatation persists in the normal but not in the sympathectomized calf.

and Swan (10) are presented in Table I. Of the sympathectomized forearms the maximum rise in flow exceeded 10 ml. in eight out of ten instances, compared with three out of ten of the normally innervated group.

Likewise, with respect to the calves, the peak flow in five of the seven sympathectomized limbs was greater than 10 ml., while in only three of the ten did flow rise above 10 ml. in the normally innervated group.

TABLE II

*Summary of second phase changes in normal and sympathectomized forearms and calves during intravenous infusions of 10μg. epinephrine per min.**

No.	Condition	Mean blood flow A (ml./100 ml./min.)	B	B−A	$\frac{B-A}{A}$ %
	Forearms				
12	Normal	2.4	5.1	2.7	122%
11	Sympathectomized	4.1	4.2	0.1	17%
	Calves				
15	Normal	3.5	5.7	2.2	73%
7	Sympathectomized	3.5	3.0	−0.5	−12%

* A is mean during the four minutes prior to start of epinephrine; B is mean during last four minutes of epinephrine infusions.

It is thus apparent that the vasodilatation in forearm and calf at the beginning of intravenous epinephrine infusions was usually greater in the sympathectomized than in the normally innervated limbs.

4. *Analysis of group responses during the second phase.* The present observations have been added to the larger series of Duff and Swan (10), and summarized in Table II. The mean control blood flow was greater in the sympathectomized than in the normally innervated group of forearms. The difference may be attributed to chance variations of small numbers of observations, but its occurrence made desirable an analysis of the influence of the control rate of blood flow on the response to epinephrine (Table III).

TABLE III

Analyses of linear regression of forearm and calf blood flow during second phase of epinephrine infusions (B), on resting blood flow (A)†*

	B estimated to equal:	Significance of coefficient	If A is 3.0 B will be:	Estimated $\frac{B-A}{A}$ %
Forearms:				
Normal	2.8 + .967A	P < .05 Sig.	5.7	90%
Sympathectomized	2.6 + .389A	P < .05 Sig.	3.8	27%
Calves:				
Normal	2.6 + .891A	P < .05 Sig.	5.3	77%
Sympathectomized	1.2 + .510A	P < .01 Sig.	2.7	−10%

* A is mean blood flow in ml./100 ml./min. during four minutes prior to start of epinephrine; B is mean blood flow in ml./100 ml./min. during last four minutes of epinephrine infusions.

† This table shows the behavior of the various groups after adjustment for inequalities of resting blood flow.

When the blood flow means were adjusted by regression analysis for inequality of the resting values, the difference between normal and sympathectomized forearms during the latter part of the intravenous epinephrine infusions remained statistically significant.

As previously shown (10), during this period (last four minutes of the epinephrine) sympathectomized limbs generally fail to exhibit normal vasodilatation. In the forearms a slight, but not significant, elevation of 17 per cent was present in the sympathectomized group ($t = 2.1$, $P = 0.8$), compared with 122 per cent in the normal group. However, in the sympathectomized calves not merely was the normal second phase of dilatation shown to be absent, but there was actually a small but significant reduction in mean flow ($t = 2.45$, $P < 0.05$).

DISCUSSION

It has been established (9, 10, 15, 16) that the initial vasodilatation in muscular segments of the limbs during infusions of epinephrine results from the direct action of epinephrine on the muscle vessels. The present finding that the magnitude of this initial increase in flow is greater in sympathectomized than in normal limbs is therefore direct evidence that sympathectomy increases the sensitivity of muscle vessels to this action of epinephrine.

The conclusion of Stein, Harpuder, and Byer (8) that hypersensitivity of the calf vessels occurs in limbs sympathectomized by preganglionic section is confirmed. A similar phenomenon is now shown to occur in sympathectomized forearms.

Concerning the second (late) phase of the response of muscle vessels during intravenous epinephrine infusions, it had already been shown that the normal dilatation was reduced or absent in sympathectomized limbs (10). In several sympathectomized individuals the blood flow, especially in the calf, was reduced below the control level, indicating actual vasoconstriction (Table II).

Direct infusions into the brachial and femoral arteries of epinephrine in amounts calculated to be somewhat higher and lower than that distributed through these vessels during intravenous infusion of 10 μg. per min. produce vasodilatation in the forearm and calf only during the first phase (10, 15, 17). During the second phase the blood flow is either at or slightly below the control level. Increasing the epinephrine concentration in intra-arterial infusions tends to cause greater vasoconstriction during this second phase (10).

The sustained moderate vasodilatation in normally innervated forearms and calves during the latter part of the intravenous epinephrine infusions was therefore due to some non-local effect of the epinephrine (10). This could have been produced by the intravenous epinephrine in three ways: *a*) elevation of systemic arterial blood pressure; *b*) neurogenic sympathetic reflexes; and *c*) liberation into the circulation of a vasodilator substance or substances.

The pressor effect of intravenous epinephrine in the concentration of 10 μg. per min. was shown to be insufficient to account for more than a small proportion of the increased blood flow during this period (10). Whelan's recent demonstration that the second phase of dilatation still occurred in forearms in which the vasomotor nerves were paralyzed by nerve block renders the neurogenic theory untenable (15). He has adduced evidence suggesting that some (as yet unidentified) humoral mechanism may be responsible for the phenomenon (15). If this be so then the inhibition or reversal of the normal second phase dilatation in the sympathectomized limbs can be satisfactorily explained only in terms of an increased sensitivity of the muscle vessels to the direct constrictor action that is normally revealed only by direct intra-arterial infusions of relatively high concentrations of epinephrine. Figures 1 and 2 indicate that in individuals with sympathectomized extremities the systemic dilator mechanism (whether humoral or otherwise) was in operation, for the normally innervated limbs obtained good second phase dilatation.

In respect to the type of operation, all the calves were sympathectomized by preganglionic decentralization, while six of the 11 forearms were sympathectomized by postganglionic denervation. There seems little doubt therefore that preganglionic and postganglionic sympathectomies are both capable of causing hypersensitivity of muscle vessels. Because of the small number of cases,

valid comparison of the two types of operation was not possible. However, the results do suggest that the degree of sensitization of the mixed group of forearms was no greater (indeed perhaps less) than that of the decentralized calves. Precise elucidation of this matter must await further comparable studies of a larger number of cases.

It has hitherto been assumed (18) that the consistently better clinical results with sympathectomy of lower than of upper limbs were largely due to the reduced liability to hypersensitivity following lumbar sympathectomy, because this operation produces decentralization rather than denervation of the vessels. There is little doubt that the treatment of vasospasm by surgical sympathectomy is more beneficial in the lower than in the upper limb, but in the opinion of many practising surgeons (19, 20) this advantage has not been overcome by adoption of preganglionic operations for the upper limb.

SUMMARY

1. The initial vasodilatation in the forearm and calf during intravenous infusions of 10 μg. epinephrine per min. has been shown to be greater in sympathectomized than in normally innervated limbs.

2. Reasons are given for attributing this increase to hypersensitivity of the vessels in muscular segments of the sympathectomized limbs.

3. The reduction or reversal of the normal second phase of vasodilatation in sympathectomized forearms and calves during intravenous epinephrine infusions has been interpreted as further evidence of vascular hypersensitivity in sympathectomized muscles.

4. These phenomena have been shown to occur in limbs sympathectomized either by preganglionic section or by ganglionectomy.

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CONCERNING THE EFFECTS OF MAGNESIUM SULFATE ON RENAL FUNCTION, ELECTROLYTE EXCRETION, AND CLEARANCE OF MAGNESIUM [1]

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The effect of the parenteral administration of magnesium sulfate on the renal hemodynamics of normal dogs (1) and normal children (2) has been previously reported. However, no data are available concerning the effects of magnesium sulfate on the excretion of other electrolytes, nor have studies on the renal clearance of magnesium been reported. It is the purpose of this report to present such data.

METHODS

Studies of renal function, electrolyte excretion, and the clearance of magnesium were performed on six young men without evidence of cardiovascular or renal disease. The experimental subjects received a normal hospital diet which contained between 7 and 15 grams of sodium chloride daily. The experiments were performed in the basal state after adequate hydration by the oral ingestion of one liter of water 60 to 90 minutes before the study. Renal plasma flow was determined by the clearance of para-aminohippurate according to the method of Goldring and Chasis (3) and utilizing a constant injection syringe as previously described (4). The glomerular filtration rate was determined by the clearance of inulin utilizing the resorcinol method of Roe, Epstein, and Goldstein (5). Following a 45 minute equilibration period, three control observations of 15 minutes each were performed. Ten cc. of 25 per cent magnesium sulfate were then added to the sustaining solution and administered by constant intravenous injection. The magnesium sulfate was administered at the rate of 1.02 milli-equivalents of magnesium per minute. After a 45 minute period, the studies of renal function, electrolyte excretion, and magnesium clearance were repeated.

Sodium and potassium were determined by an internal standard Perkin-Elmer flame photometer. Control studies indicated that concentrations of magnesium exceeding those found in the urine in these experiments had no effect on the flame photometric determination of sodium or potassium. Chloride was determined by the method of Schales and Schales (6). Serum magnesium was determined by the molybdivanadate method of Simonsen, Westover, and Wertman (7). Urinary magnesium was determined by a similar method as modified by Stutzman (8).

RESULTS

The control values for renal hemodynamic, electrolyte excretion, and magnesium clearance studies are the averages of three consecutive periods. The values during the constant intravenous administration of magnesium sulfate are the averages of three consecutive periods beginning 45 minutes after the start of the infusion.

There was a significant decrease in mean renal plasma flow ($t = 3.45$, p 0.02—0.01) and in glomerular filtration rate ($t = 5.98$, $p < 0.01$). Although the mean percentage change was slight, being 9.9 per cent for renal plasma flow and 8 per cent for glomerular filtration rate, both functions decreased in each patient. Since the statistical analysis was done by the method of paired controls $\left(t = \frac{\bar{x}}{\sqrt{\frac{s^2}{n}}}\right)$ and since the changes were all in the same direction, these changes in renal plasma flow and glomerular filtration rate are statistically significant. However, the degree of change does not imply physiologic significance. The filtration fraction increased in three patients and decreased in three. The clearance data are recorded in Table I.

The serum sodium, potassium, and chloride concentrations were unchanged by the administration of magnesium sulfate. An increase in the urinary sodium and chloride excretion and a decrease in potassium excretion occurred in each patient and

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TABLE I

Renal hemodynamic studies before and during magnesium sulfate infusion

Patient	Renal plasma flow (*cc. per min.*)		Glomerular filtration rate (*cc. per min.*)		Filtration fraction (*per cent*)	
	Control	During magnesium sulfate	Control	During magnesium sulfate	Control	During magnesium sulfate
J. G.	705	567	123	113	17.4	19.9
P. M.	614	556	130	119	21.1	21.0
H. C.	769	663	138	134	17.9	20.2
L. S.	852	832	141	132	16.5	15.8
R. S.	574	563	110	100	19.1	17.5
D. D.	723	638	116	99	14.6	15.5
Mean	706.2	636.5	126.3	116.2	17.7	18.3
Per cent change		−9.9		−8		+3.4

the mean changes were statistically significant (sodium, $t = 3.64$, p 0.02—0.01; chloride, $t = 3.32$, p 0.05—0.02; potassium, $t = 4.78$, $p < 0.01$).

ministration of magnesium sulfate. The data for urinary electrolyte excretion are noted in Table II.

The mean control serum magnesium concen-

TABLE II

Urine electrolyte excretion before and during magnesium sulfate infusion

Patient	Urine sodium excretion (*micro-equivalents per min.*)		Urine chloride excretion (*micro-equivalents per min.*)		Urine potassium excretion (*micro-equivalents per min.*)	
	Control	During magnesium sulfate	Control	During magnesium sulfate	Control	During magnesium sulfate
J. G.	302	431	332	415	110	77
P. M.	242	380	160	288	80	51
H. C.	197	423	177	362	110	66
L. S.	211	235	175	213	118	51
R. S.	197	299	205	279	79	55
D. D.	192	230	202	210	147	62
Mean	223.5	333	208.5	294.5	107.3	60.3
Per cent change		+48.9		+41.2		−43.8

Analysis of urinary electrolyte excretion in the individual periods revealed that the changes began in the first period of observation following the ad-

tration was 1.64 mEq. per liter. The clearance of magnesium was calculated from the usual clearance formula. The mean control value for the

TABLE III

Renal clearance of magnesium

Patient	Serum magnesium (*mEq. per liter*)				Renal clearance of magnesium (*cc. per min.*)		Clearance magnesium / Clearance inulin ratio	
		During magnesium administration						
		Period						
	Control	4	5	6	Control	During magnesium administration	Control	During magnesium administration
J. G.	1.69	2.18	2.45	2.60	6.36	18.2	.052	.161
P. M.	1.52	2.18	2.32	2.40	6.75	26.0	.052	.218
H. C.	1.49	2.07	2.23	2.41	3.98	31.8	.029	.237
L. S.	1.73	2.24	2.34	2.49	4.65	32.5	.033	.246
R. S.	1.68	2.19	2.32	2.35	5.79	24.2	.053	.242
D. D.	1.73	2.42	2.58	2.68	4.69	25.2	.040	.254
Mean	1.64	2.21	2.37	2.49	5.37	26.3	.043	.226

clearance of magnesium was 5.37 cc. per minute. The ratio of the clearance of magnesium to that of inulin was 0.043 during the control period, yielding a mean value for magnesium tubular reabsorption of 95.7 per cent. The serum magnesium concentration did not remain constant after the magnesium sulfate infusion was started. The mean values for the three post-magnesium periods were 2.21, 2.37, and 2.49 mEq. per liter. In view of these increasing serum concentrations, the clearance of magnesium during these periods could only be approximated. The mean approximate clearance for the last three periods was 26.3 cc. per minute. The mean ratio of magnesium to inulin clearance for these periods was 0.226 and mean tubular reabsorption of magnesium decreased to 77.4 per cent. The average urinary excretion of magnesium was 8.5 micro-equivalents per minute during the control periods and 64.6 micro-equivalents per minute during the infusion of magnesium sulfate. The data concerning magnesium are recorded in Table III.

DISCUSSION

The present concept of the action of magnesium sulfate upon the kidney is that the renal vascular bed participates in the generalized vasodilatation which follows the parenteral administration of magnesium (1, 2). Thus renal plasma flow and glomerular filtration rate may be maintained despite a drop in blood pressure, these results being explained by renal afferent arteriolar dilatation. The validity of this concept is supported by the observation that magnesium sulfate produces renal hyperemia, without significant changes in the glomerular filtration rate, in the hypertensive phase of acute glomerulonephritis (9).

It is to be noted that our experimental procedure differed from those previously reported since our control studies and studies during the administration of magnesium sulfate were performed consecutively. The total duration of each experiment was three to three and one-half hours. Under these experimental conditions, the intravenous administration of magnesium sulfate was associated with a slight but statistically significant decrease in renal plasma flow and glomerular filtration rate. No significant change in blood pressure was noted in any of the subjects.

The action of magnesium sulfate in causing an increase in the urinary excretion of sodium and chloride is of interest. Increased load is obviously not a factor since the serum concentration of these ions did not change and since there was actually a decrease in the glomerular filtration rate. Osmotic activity by the magnesium ion could not have played a role since the rate of urine excretion decreased in most of the experimental periods following the magnesium infusion. It is further to be noted that the excretion of potassium decreased at a time when sodium and chloride excretion were increasing. The mechanism responsible for these changes is unknown. The data presented are compatible with the hypothesis that magnesium, a divalent ion, may enter into a univalent ion exchange mechanism resulting in a decreased tubular reabsorption of sodium and increased reabsorption of potassium. However, the evidence is indirect, and it is possible that other mechanisms are involved.

It has been suggested that magnesium is excreted by the renal tubules (10). However, there are no previous observations in the literature relative to the clearance of magnesium. While the clearance of magnesium increases rapidly with increased magnesium loading, the values for urinary excretion in these experiments are far below those required to implicate tubular excretory processes as a mechanism in the renal clearance of magnesium. For example, at a serum magnesium concentration of 2.49 mEq. per liter and a glomerular filtration rate of 116 cc. per minute, 289 micro-equivalents per minute would be excreted by filtration alone assuming no tubular reabsorption. At comparable values in our experiments, 64.6 micro-equivalents per minute were excreted. In order to test the hypothesis that tubular excretion of magnesium occurs, it would be necessary to increase the magnesium loading to levels that would probably be unsafe in human experiments. Since it has been demonstrated that magnesium is excreted by the aglomerular fish kidney (11), the possibility of the tubular excretion of magnesium by the human kidney must remain an open question. Experimental observations in patients with renal insufficiency and in animals, similar to those involving potassium excretion, may provide the answer.

SUMMARY AND CONCLUSIONS

The constant, intravenous administration of magnesium sulfate to subjects without cardiovascular or renal disease was associated with a slight but significant decrease in renal plasma flow and glomerular filtration rate. There was an increased urinary excretion of sodium and chloride and a decreased excretion of potassium. The serum concentration of these electrolytes did not change. The mean renal clearance of magnesium in the basal state was 5.37 cc. per minute. This increased to 26.3 cc. per minute following the injection of magnesium. The data presented suggest that magnesium may be involved in an ion exchange mechanism.

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PRELIMINARY ELECTROPHORETIC ANALYSIS OF HUMAN GASTRIC JUICE [1, 2]

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In a recent publication (1) electrophoretic data were reported on two mucoproteins obtained by fractionation of human gastric juice according to the method of Glass and Boyd (2). One of the separated components, "glandular mucoprotein," contains an acid mucopolysaccharide as a prosthetic group, while the other separated mucoprotein, termed "mucoproteose," contains a neutral polysaccharide as a prosthetic group. Both mucoproteins were found to be fairly homogeneous electrophoretically and both were negatively charged in the pH range studied (5.0 to 8.5); however, the "glandular mucoprotein" migrated with an exceptionally high mobility and the "mucoproteose" with an exceptionally low mobility. The present study was undertaken to ascertain whether or not the electrophoretic data reported on these two chemically fractionated mucoproteins could be correlated with the electrophoretic pattern of whole human gastric juice.

METHOD

Fasting gastric juice was obtained by intubation of healthy volunteers and patients with peptic ulcer. The subjects were urged not to swallow saliva. Specimens discolored by bile were discarded. The gastric juice collected was then treated by one of the following methods: *Method A:* Freshly collected juice was filtered, neutralized to a pH of approximately 7 by adding dropwise, with twirling, 1 *N* sodium hydroxide, dialyzed for 24 hours against cold (5° to 10° C.) running tap water, dialyzed for 24 hours against cold (2° to 5° C.) distilled water, and then dried by lyophilization. *Method B:* Immediately after collection, the gastric juice was placed in a refrigerator maintained at 2° to 5° C. At a later time, varying from a few hours to several days, the gastric juice was filtered, dialyzed against cold running tap water and distilled water, and lyophilized.

The prepared material was then dissolved in buffer. Both phosphate and veronal buffers were used; an ionic strength of 0.1 M was maintained in all experiments. In the initial experiments the concentrations of the protein solutions varied from 1 per cent to 2 per cent; in later experiments concentrations of 2.5 per cent to 5 per cent were used. At these higher concentrations the protein solutions were turbid but could be cleared by centrifuging for 15 minutes at 15,000 to 20,000 RPM. It is recognized that this maneuver may remove some of the less soluble components but loss of some material would not vitiate the inferences drawn from this study. All protein solutions were dialyzed against the buffer used through Visking tubing tied off at both ends; if this precaution was not taken the solutions within the tubing swelled and consequently became more dilute. Specimens of saliva were immediately filtered, dialyzed against cold running tap water and cold distilled water, and lyophilized. This material was then dialyzed against the buffer prior to electrophoresis. The majority of the *electrophoresis experiments* were carried out with an Aminco portable electrophoresis apparatus and a clinical cell. A few additional experiments were performed with the aid of an Aminco-Stern electrophoresis apparatus and an analytical cell, and with a Klett electrophoresis apparatus and an analytical cell. In keeping with custom, mobilities were calculated from the descending limb and expressed in units $\times 10^{-5}$ cm.^{2} sec.$^{-1}$ volts^{-1}. Relative concentration was grossly estimated on the basis of planimeter measurements of the peaks in the ascending limb.

RESULTS

I. Electrophoretic Patterns

1. *Electrophoretic patterns at varying values of pH.* The results of a series of experiments on a single preparation, obtained by Method B, at four different pH levels and at a potential gradient of from 5.1 to 5.5 volts per cm., are listed in Table I. These experiments were carried out with a Klett electrophoresis apparatus and an

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analytical cell. Although the patterns were essentially the same at all pH levels, the best resolution was achieved at the highest pH value (8.5). The electrophoretic pattern of the experiment performed with 0.1 ionic strength phosphate buffer and at a pH of 6.1 and a protein concentration of 1.4 per cent is shown in Figure 1. To facilitate discussion the various peaks are arbitrarily identified by symbols, corresponding to those used in Table I.

An inspection of Table I reveals that the components migrate at a higher mobility in phosphate buffer of pH 6.9 than in veronal buffer of pH 8.5. This anomalous result was also observed by Pugh, Glass and Wolf (1) in their electrophoretic studies of the fractionated gastric mucoproteins. On the basis of the results listed in Table I, all ensuing electrophoresis experiments were carried out in 0.1 ionic strength veronal buffer of pH 8.5 ± 0.1. These conditions were deemed the best for analysis to achieve optimal resolution and greatest protein concentration (the solubility was greater at a higher pH).

2. *Patterns in the gastric juice of healthy subjects.* Six electrophoresis experiments on material obtained from the gastric juice of healthy subjects were carried out in veronal buffer at a hydrion concentration of 8.5 ± 0.1 and an ionic strength of 0.1. The protein concentrations varied from 1.1 per cent to 5.0 per cent. The results are listed in Table II which indicates the range of mobilities and the concentrations roughly estimated from planimetric measurements of the peaks.

3. *Patterns in the gastric juice of patients with duodenal ulcer.* Fourteen experiments were performed on material obtained from the gastric juice of patients with duodenal ulcer. These were run at the same pH and in the same range of protein concentration as the observations on healthy subjects. The results are listed in Table II where in comparison with the healthy subjects it is evident that the electrophoretic pattern is the same as regards mobility but among the ulcer patients the concentration of the G-1 component appears to be significantly greater and the concentration of the G-3 component significantly smaller than among the healthy subjects.

4. *Electrophoretic pattern of saliva.* Because it was virtually impossible to prevent some degree of salivary contamination during the collection of the gastric juice, it seemed important to determine the electrophoretic pattern of saliva. Figure 2 presents the electrophoretic pattern of pooled human saliva after migration for 12,720 seconds in 0.1 M veronal buffer at pH 8.5 and in protein concentration of 3.0 per cent. The mobilities (calculated from the descending limb) of the S-1 and S-2 components are -4.2×10^{-5} and -2.4×10^{-5} cm.^{2} sec.$^{-1}$ volts^{-1}, respectively. These mobilities are of the same order of magnitude as the G-2 group of proteins. In addition, the resolution of the S-2 component of this saliva preparation into two components on the ascending limb is very similar to that of the G-2a component of the gastric juice preparation.

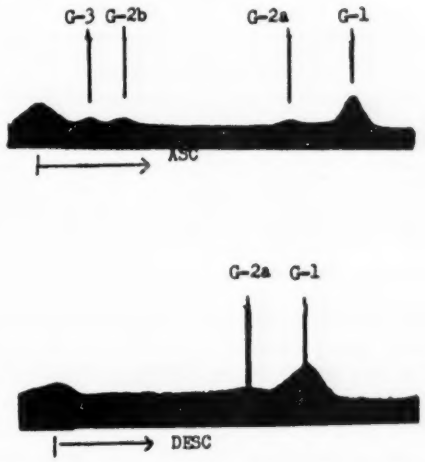

FIG. 1. THE ELECTROPHORETIC PATTERN OF GASTRIC JUICE FROM A HEALTHY SUBJECT IN 0.1 IONIC STRENGTH PHOSPHATE BUFFER AT PH 6.0 AND A PROTEIN CONCENTRATION OF 1.4 PER CENT

TABLE I

Mobilities of components at varying hydrion concentrations

Buffer	pH	Protein conc. %	Mobility (Desc. limb)*			
			G-1	G-2a	G-2b	G-3
Phosphate	5.1	1.6	6.2	4.3	†	†
Phosphate	6.1	1.4	8.9	6.8	†	†
Phosphate	6.9	1.6	9.8	7.7	1.2	.7
Veronal	8.5	1.4	7.8	4.7	1.6	.8

* Mobilities expressed in units of $-u \times 10^{-5}$ cm.^{2} sec^{-1} volts^{-1}.

† Component not resolved.

TABLE II

Range of mobility calculated from experiments on material from the gastric juice of normal individuals and patients with duodenal ulcer

Component	Controls		Ulcer patients	
	Mobility	Relative conc.	Mobility	Relative conc.
G-1	7.4–8.4	5–15	7.2–8.4	10–25
G-2a	3.5–5.5	20–50	3.3–5.7	10–25
G-2b	1.0–2.0	5–10	1.0–2.0	5–10
G-3	0.4–1.0	20–40	0.4–1.0	5–15

The possibility that the G-2 group proteins in gastric juice represented solely contaminating saliva was ruled out by finding a small diffuse G-2 peak, of a size comparable to that seen in the gastric juice of intact subjects, in the gastric juice of Tom, an individual with a gastric fistula and an occluded esophagus (3). The ascending limb of the electrophoretic pattern, after 5,070 and 9,810 seconds, is shown in Figure 3; the mobilities of the G-1, G-2, and G-3 components were −8.4, −4.7, and -1.0×10^{-5} cm.^{2} sec.$^{-1}$ volts^{-1}, respectively.

II. Attempts to Identify "Glandular Mucoprotein" and "Mucoproteose" in the Electrophoretic Pattern of Human Gastric Juice

One gram of material prepared by Method B was dissolved in veronal buffer of pH 8.6, and electrophoresis determinations were carried out on the first aliquot of this solution alone, on a second aliquot to which "glandular mucoprotein" had been added, and on a third aliquot to which "mucoproteose" had been added. The amounts

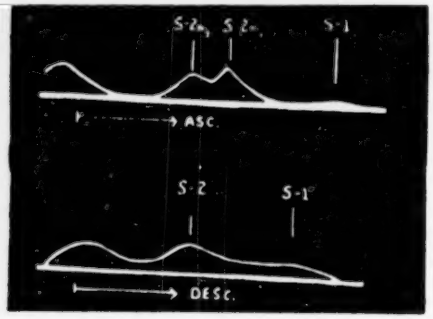

FIG. 2. THE ELECTROPHORETIC PATTERN OF HUMAN SALIVA IN A 0.1 IONIC STRENGTH VERONAL BUFFER AT pH 8.5 AND A PROTEIN CONCENTRATION OF 3.0 PER CENT

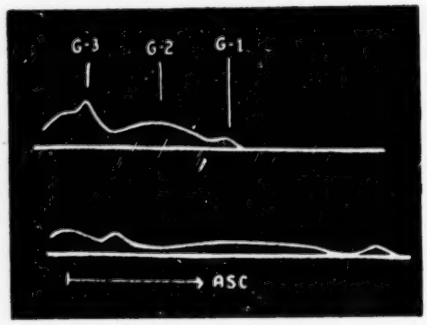

FIG. 3. THE ELECTROPHORETIC PATTERN OF THE GASTRIC JUICE OF TOM UNCOMTAMINATED BY SALIVA SHOWN IN THE ASCENDING LIMB AFTER 5,070 AND 9,810 SECONDS

and relative concentrations of the lyophilized gastric proteins (L.G.P.), the "glandular mucoprotein" (MPN), and the "mucoproteose" (MPTS) in each solution are tabulated in Table III.

The electrophoretic pattern of the plain gastric specimen is shown in Figure 4, upper tracing. It is a typical pattern, consisting of a fairly homogeneous leading component, G-1; a very inhomogeneous, diffuse center component, G-2a; a slight amount of protein forming a fairly slow peak, G-2b; and a fairly homogeneous, slowest moving component, G-3. The added MPN formed a peak about midway between components G-1 and G-2a (Figure 4, middle tracing). The added MPTS increased the area of the slowest moving component, G-3 (Figure 4, lower tracing). The mobilities of the components (calculated from the descending limb) are given in Table IV.

The MPN analyzed in a separate experiment at a concentration of 1.8 per cent migrated as a single component and appeared more homogene-

TABLE III

Amounts and relative concentrations of protein in the plain gastric specimen (A) and in those to which "glandular mucoprotein" (B) and mucoproteose (C) had been added

Expt. no.	Mg. of L.G.P.	Mg. of MPN added	Mg. of MPTS added	Total prot. conc.* %	Conc. of components		
					L.G.P. %	MPN %	MPTS %
A	402	—	—	2.9	2.9	—	—
B	268	134	—	3.0	2.0	1.0	—
C	268	—	134	3.0	2.0	—	1.0

* Total protein concentration was calculated by dividing total dry weight of all protein material by the total volume of protein solution after dialysis.

ous than the MPN peak of Experiment B. The mobility of the MPN in this experiment was -6.9×10^{-5} cm.^{2} sec.$^{-1}$ volts^{-1}, as compared to the mobility of -5.5×10^{-5} in Experiment B. The MPTS analyzed in a separate experiment at a concentration of 1.5 per cent migrated as a single component, with a mobility of -0.8×10^{-5} cm.^{2} sec.$^{-1}$ volts^{-1}.

III. Electrophoretic Analysis of Protein Preparations Obtained by Fractionation of Gastric Juice According to the Method of Glass and Boyd (2)

Bile-free gastric juice from a fasting patient with duodenal ulcer was collected and filtered at twenty minute intervals over a three hour period. This gastric juice was then divided into three equal portions and fractionated as follows: Fraction 1 was obtained by treating one portion by method as described above. Fraction 2 was obtained by treating a second portion by the first step in the Glass and Boyd method (2); *i.e.*, removal of contaminants by precipitation with trichloracetic acid, and then was dialyzed and lyophilized. Fraction 3 was obtained by treating a third portion by the first two steps in the Glass and Boyd method, *i.e.*, removal of contaminants with trichloracetic acid and precipitation of "mucin" with acetone. The "mucin" was then dissolved in 0.1 N sodium hydroxide, was dialyzed, and then lyophilized.

An electrophoresis experiment was performed on each of the fractions obtained in the manner described above. The Aminco portable electrophoresis apparatus and clinical cell were employed, and the analyses were carried out using 0.1 ionic

TABLE IV

Mobilities of components in the plain gastric specimen (A) in those to which glandular mucoprotein (B) and mucoproteose (C) had been added

Component	Mobility ($-u \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volts^{-1})		
	Exp. A	Exp. B	Exp. C
G-1	7.4	7.5	7.4
G-2a	4.3	*	*
G-2b	1.0	1.2	†
G-3	.5	.7	.6
MPN	—	5.5	—

* Component too diffuse for accurate calculation.
† Component missing, probably obscured by large MPTS peak.

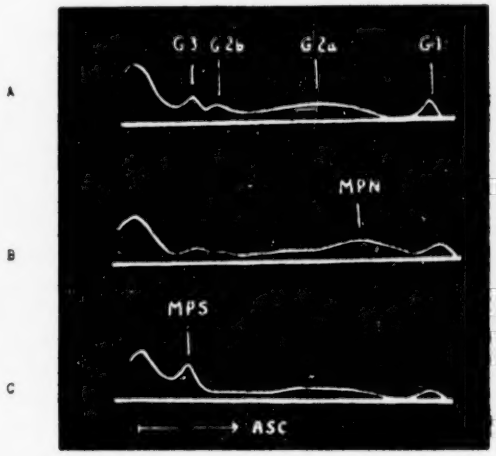

FIG. 4. A: THE ELECTROPHORETIC PATTERN OF GASTRIC JUICE FROM A HEALTHY SUBJECT IN 0.1 IONIC STRENGTH VERONAL BUFFER AT pH 8.6 AND A PROTEIN CONCENTRATION OF 3.9

B: THE SAME AFTER ADDITION OF GLANDULAR MUCOPROTEIN

C: THE SAME AS A AFTER ADDITION OF MUCOPROTEOSE

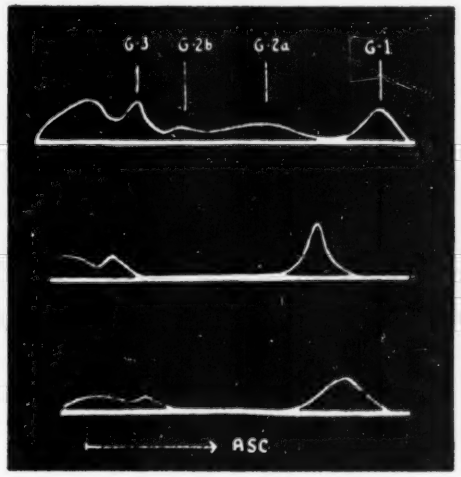

FIG. 5. A: THE ASCENDING LIMB OF THE ELECTROPHORETIC PATTERN OF HUMAN GASTRIC JUICE

B: THE SAME AFTER TREATMENT WITH TRICHLORACETIC ACID

C: THE SAME AS A AFTER TREATMENT WITH BOTH TRICHLORACETIC ACID AND ACETONE

TABLE V

Mobilities of protein components in fractions separated by method of Glass and Boyd

Fraction	$-u \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volts^{-1}			Protein conc.* %
	G-1	G-2	G-3	
1. Plain gastric specimen	7.7	4.7	1.5	2.5
2. Treated with trichloracetic acid	5.5	0	.4	2.4
3. Treated with trichloracetic acid and acetone	7.1	0	1.8	1.8

* Protein concentration calculated on basis of dry weight of lyophilized material, with assumption that all material is protein in nature.

strength veronal buffer of pH 8.5. The ascending limb electrophoretic patterns of the three fractions are shown in Figure 5, and the mobilities of the designated components (calculated from the descending limb) are tabulated in Table V.

The electrophoretic pattern of whole gastric juice proteins (Fraction 1) exhibited five maxima in the refractive index gradient curve, one of them representing the stationary boundary (Figure 5), upper tracing. The component of highest mobility, G-1, was fairly homogeneous and represented 14 per cent of the total concentration of material. The mobility of this G-1 protein was of the same order of magnitude in Fractions 1, 2, and 3. However, in Fraction 2 it represented 35 per cent and in Fraction 3, 40 per cent of the total concentration. In addition, the peak was sharper and more symmetrical in the electrophoretic diagram of Fraction 2 than in either Fraction 1 or 3.

COMMENT

It is as yet impossible to state whether the change in mobility and symmetry of the G-1 component is due to denaturation of the proteins during the fractionation procedure, to the action of proteolytic enzymes present in gastric juice, or to some other cause. At the present time, studies are in progress to obtain pure components by utilizing a modification of the low temperature-ethanol fractionation technique used by Cohn and his associates in the fractionation of blood (4).

The G-2 group proteins were evident in the electrophoretic pattern of Fraction 1; their mobilities were of the same order as previously calculated. However, in the electrophoretic patterns of Fraction 2 and 3 no peaks corresponding to the G-2 group proteins were evident. Glass and Boyd have demonstrated (2) that the trichloracetic acid removes contaminating salivary proteins, and since it has been shown above that salivary proteins are related to the G-2 group proteins, the absence of any G-2 group in the electrophoretic patterns of Fractions 2 and 3 is not altogether surprising. However, it should be stressed that the trichloracetic acid removes the G-2 proteins of gastric origin as well as those of salivary origin.

The relative concentration of the G-3 protein, as determined from the electrophoretic diagrams, was virtually identical for Fractions 1, 2, and 3. In addition, in all three fractions the mobilities of this component were of the same order of magnitude and the symmetry was essentially the same.

DISCUSSION AND SUMMARY

The electrophoretic analyses of the concentrated gastric juice of normal individuals and of patients with duodenal ulcer demonstrated the presence of at least five different proteins whose relative concentrations varied considerably.

It is recognized that the procedures required in the preparation of the material for electrophoretic analysis may have denatured or in some way altered the electrical properties of the proteins. The component with the highest mobility was designated as G-1. Fairly homogeneous, it occurred in higher concentration in the gastric juice of patients with duodenal ulcer than in that of normal individuals. Glass, Boyd, and Svigals (5) had earlier found a higher concentration of "glandular mucoprotein" (MPN) in juice from individuals with duodenal ulcer than from healthy subjects. This correlation, plus the results of the fractionation of gastric juice by the Glass and Boyd method reported above, suggests that the G-1 component is related to glandular mucoprotein. The fact that prepared "glandular mucoprotein" when added to lyophilized gastric juice protein migrated as a separate component, suggests that the glandular mucoprotein is a form of the G-1 component denatured by the drastic conditions of its preparation from a 60 per cent acetone solution at room temperature.

The resolution, mobilities, and relative concentrations of the group of proteins designated as the G-2 components varied considerably, with the

number of separate peaks varying from one to three. The peaks of these proteins were diffuse, indicating inhomogeneous, low molecular weight material. Although it was demonstrated that contaminating salivary proteins contribute to the G-2 group components, the experiment on the juice of the gastric fistulous subject with occluded esophagus suggests that gastric juice proteins also contribute to this group.

The peak designated as the G-3 component indicated the presence of a fairly homogeneous protein, migrating at a very low mobility. This component was electrophoretically identical with "mucoproteose" (MPTS) of Glass and Boyd. The studies of material partly fractionated by the Glass and Boyd method suggest that the mucoproteose concentration, as determined by chemical analysis, includes in part dialyzable, low molecular weight material. This fraction of mucoproteose could, of course, not be examined electrophoretically.

CONCLUSIONS

With the limitations in mind that the preparatory procedures may have altered the proteins, it may be stated that the electrophoretic pattern of whole human gastric juice reveals the presence of at least five electrophoretically distinct proteins, present in varying concentrations. The component of highest mobility seems to be related to, but is not identical with, the "glandular mucoprotein" of Glass and Boyd. The component of lowest mobility is electrophoretically identical with the "mucoproteose" of Glass and Boyd.

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EVIDENCE IN MAN THAT URINARY ELECTROLYTE LOSS INDUCED BY PITRESSIN IS A FUNCTION OF WATER RETENTION [1]

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INTRODUCTION

There are many conflicting reports on the acute effects of posterior pituitary extract on the renal excretion of sodium and chloride. This subject has been recently reviewed (1).

The present study was made to determine the effects on electrolyte excretion in man of a long-acting posterior pituitary extract administered over a period of several days. The results suggest that the state of hydration determines the effect of pitressin on electrolyte excretion. The relationship of the findings to the problem of volume regulation of the body fluids will be considered.

METHODS

Metabolic studies were conducted at the Metabolism Ward of the Massachusetts General Hospital (2) and at the U. S. Public Health Service Hospital, Baltimore, Maryland. Subjects studied included four normal adults, two patients with anterior pituitary insufficiency, one with anterior and posterior pituitary insufficiency, one with adrenal cortical insufficiency, and one with ovarian agenesis. Subjects were maintained on a constant diet, and the fluid intake was controlled at various levels. No restrictions were made on the activity or position of the subjects. Dietary sodium intake was fixed in each study and ranged in different studies from 20 to 175 mEq. per day. Following an initial control period of several days, posterior pituitary extract was administered intramuscularly as Pitressin Tannate in Oil,[5] 2.0 units initially, followed by 1.0 unit every 12 hours for two to four days. This dose of pitressin was estimated to be within the physiological range of endogenous antidiuretic hormone production (3, 4). Twenty separate periods of pitressin administration were studied. In all but one instance an antidiuretic effect was maintained throughout the period of hormone administration.

Urine and serum were analyzed for sodium, chloride, potassium, nitrogen, and total solutes. Two studies included calcium and phosphorus determinations and stool analyses. Analytical methods used have been described in a previous communication (5). Recently, however, the saline standard solutions used in the measurement of total solute concentrations have been corrected for activity coefficients calculated from data obtained in the International Critical Tables (6). Thus, in the studies on R. S., N. W., and O. W. in which the new standards were used, the normal level of total solute concentration of serum was found to be approximately 290 mOsm. per L. rather than the previously reported value of 310 mOsm. per L.

Inulin and para-aminohippurate clearances (7) were used as measures of glomerular filtration rate and renal plasma flow in one subject, M. K. H. In two subjects, R. S. and O. W., 24 hour clearances of endogenous creatinine were used as an estimate of glomerular filtration rate [6] (8). Acute changes in plasma volume were calculated in subjects R. S. and O. W. from hematocrit and hemoglobin measurements (9).

RESULTS

A. *The effects of pitressin on electrolyte excretion*

Figure 1 shows a typical response of a normal subject to administration of pitressin over a period

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[5] Lots L888G or L654C were used. The Pitressin Tannate in Oil was generously supplied by Dr. A. C. Bratton, Jr., Parke, Davis and Co., Detroit 23, Michigan.

[6] Although not as valid a measure of glomerular filtration rate as is the clearance of inulin, the clearance of endogenous creatinine is a useful estimate of the filtration rate over the daily period of urine collection. This avoids the fallacy of correlating the excretion of water and solutes over twenty-four hours with the clearance of inulin over a few minutes.

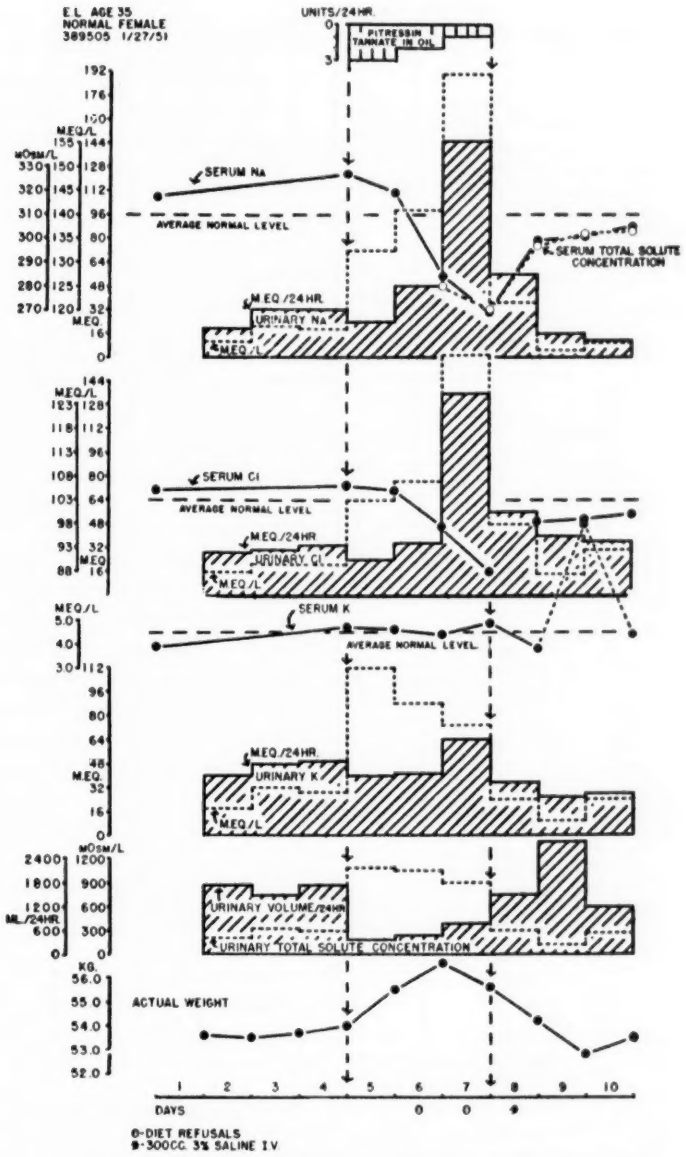

FIG. 1. THE EFFECTS OF PITRESSIN ON SERUM ELECTROLYTE CONCENTRATIONS, URINARY ELECTROLYTE EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, AND BODY WEIGHT OF A NORMAL SUBJECT

The hatched columns represent total 24 hour excretions and the broken lines urinary concentrations. Note the prompt antidiuretic effect of pitressin but the delay in the excretion of sodium and chloride. Sodium intake was 60 mEq. per day by analysis. Because of anorexia and nausea, part of the diet and fluid intake was refused on the last two days of pitressin administration. The day after pitressin was stopped 300 ml. of three per cent saline were administered intravenously to correct the extracellular fluid hypotonicity. The abnormally high serum potassium on Day 10 was twice repeated but remains unexplained; no comparable result was seen in any other experiment.

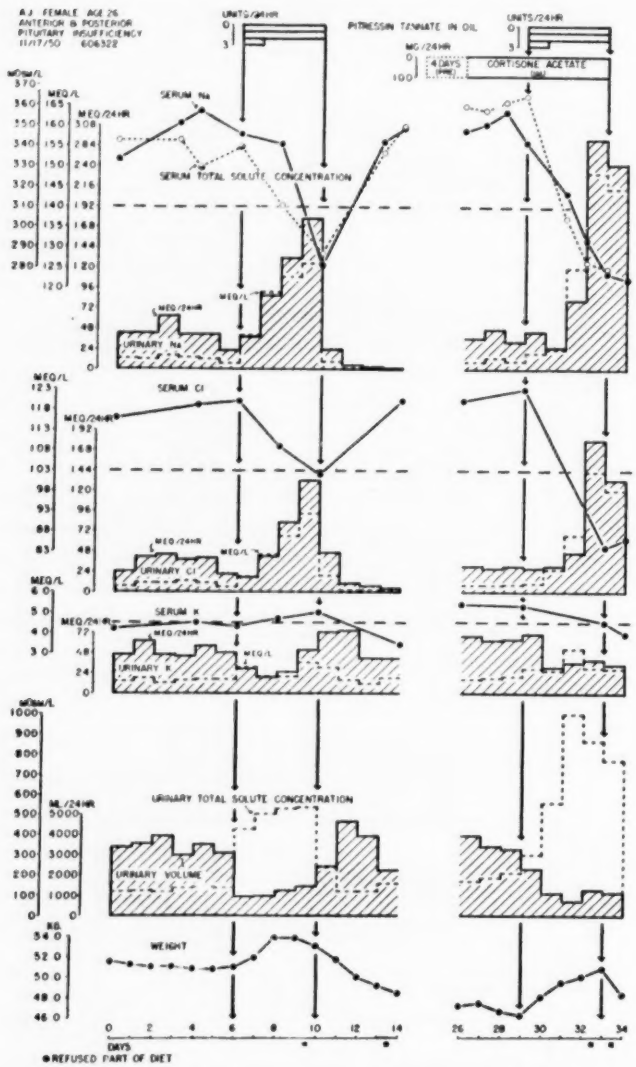

FIG. 2. THE EFFECTS OF PITRESSIN ON SERUM ELECTROLYTE AND TOTAL SOLUTE CONCENTRATIONS, URINARY ELECTROLYTE EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, AND BODY WEIGHT IN A SUBJECT WITH ANTERIOR AND POSTERIOR PITUITARY INSUFFICIENCY

The concentrations of serum sodium, chloride and total solute were abnormally high during the control period. In the left-hand section of this figure the effects of pitressin alone are presented. In the right-hand section the effects of pitressin following seven days of continuous cortisone therapy are presented. On cortisone the antidiuretic effect of pitressin was increased, as indicated by the higher urinary total solute concentration. The sodium and chloride loss and symptoms of water intoxication were also increased with cortisone. Sodium intake was 49 mEq. per day by analysis.

of three days. The antidiuretic effect of pitressin is seen in the abrupt decrease in urine volume and increase in urine total solute concentration that occurred on the first day of hormone administration. The water retention produced by this antidiuretic effect resulted in an increase in body weight and dilution of serum sodium, chloride and total solutes. On the third day of pitressin administration a large increase in urinary sodium and chloride excretion occurred. The large water diuresis following the pitressin period represented excretion of water retained during pitressin administration, and returned the weight to the control level. The urinary sodium excretion fell to low levels in the post-pitressin period. These after-effects have been constant sequelae of pitressin administration when the latter has caused fluid retention and sodium loss.

The left-hand section of Figure 2 shows similar data obtained from a patient with anterior and posterior pituitary insufficiency. Again pitressin had a prompt antidiuretic effect. Only after the first 24 hours was there an increased excretion of sodium and chloride, which progressed stepwise throughout the remainder of the pitressin period.

Similar, though less striking, results were obtained in a subject with adrenal cortical insufficiency (Figure 3) treated with 25 mgs. of cortisone acetate orally every eight hours throughout the study.

The delay in the appearance of the increases in electrolyte excretion suggested that they could be dissociated from the prompt antidiuretic action of pitressin. The increased sodium excretion during administration of pitressin was associated with progressive retention of water. This suggested that the effects on electrolyte excretion could result from the overhydration produced by pitressin rather than from a primary action of pitressin.

B. *Prevention of the effects of pitressin on electrolyte excretion by dehydration*

To test this hypothesis pitressin was given to three normal subjects during periods of low fluid intake which would preclude fluid retention in spite of exogenous antidiuretic activity. The same dose of pitressin was repeated later in the study during periods of high fluid intake. Figures 4 and 5 show the influence of the state of hydration on renal sodium excretion.

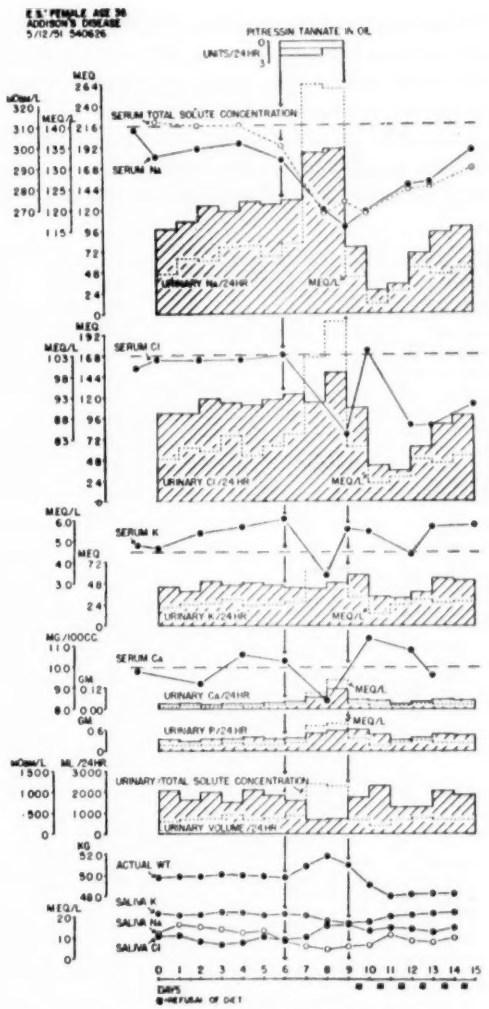

FIG. 3. THE EFFECTS OF PITRESSIN ON SERUM ELECTROLYTE AND TOTAL SOLUTE CONCENTRATIONS, URINARY ELECTROLYTE AND NITROGEN EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, AND BODY WEIGHT IN A SUBJECT WITH ADRENAL CORTICAL INSUFFICIENCY

The subject was maintained on 25 mg. of cortisone acetate orally every eight hours throughout the study. Urinary calcium and phosphorus excretion and salivary electrolyte concentrations are also shown. Sodium intake was 124 mEq. per day by analysis.

Figure 4 shows the effects of two different levels

of fluid intakes on the excretion of electrolytes during pitressin administration. The urine volume was small, and its total solute concentration high, during the control period of low fluid intake. Administration of pitressin resulted in no further increase in urine total solute concentration and no fluid retention, as indicated by the constancy of body weights and the absence of serum dilution. No change in sodium or chloride excretion resulted from pitressin in the absence of fluid retention.

The fluid intake was then increased. Urine volumes increased and urine concentration dropped. When pitressin was again given, its prompt antidiuretic effect was evident. Water was now retained, as indicated by the weight curve. A definite increase in urinary sodium was present on the third day of pitressin. The increase in chloride occurred the following day. The marked dilution of the serum sodium and total solute concentrations resulted both from water retention and, to a lesser extent, from excretion of sodium.

Figure 5 shows the effect of three different levels of fluid intake on the excretion of electrolytes during pitressin administration. Again with restriction of fluid intake to low levels (920 ml. of water in total fluid intake daily) no significant changes in sodium and chloride excretion were noted. When a further 1,500 ml. of water were given daily, repetition of the pitressin dosage now resulted in definite fluid retention and weight gain, accompanied by a slight increase in sodium and chloride excretion. Finally, the fluid intake was increased to a total of 4,000 ml. daily; pitressin now caused a very large increase in sodium and chloride excretion.[7] Because of the rapidity of water retention and the marked dilution of serum solute concentration that occurred with the high fluid intake, the last pitressin period was shortened to two days' duration.

These observations suggested that the electrolyte excretion following pitressin is determined by the state of hydration. Figure 6 shows the relationship between per cent increase in body weight and cumulative sodium loss during fifteen periods of pitressin administration. It is apparent that a rough correlation exists between fluid retention and sodium loss.

The possibility existed that the late increase in sodium and chloride excretion might simply be the result of an accumulation of pitressin activity in excess of physiological amounts. The total of

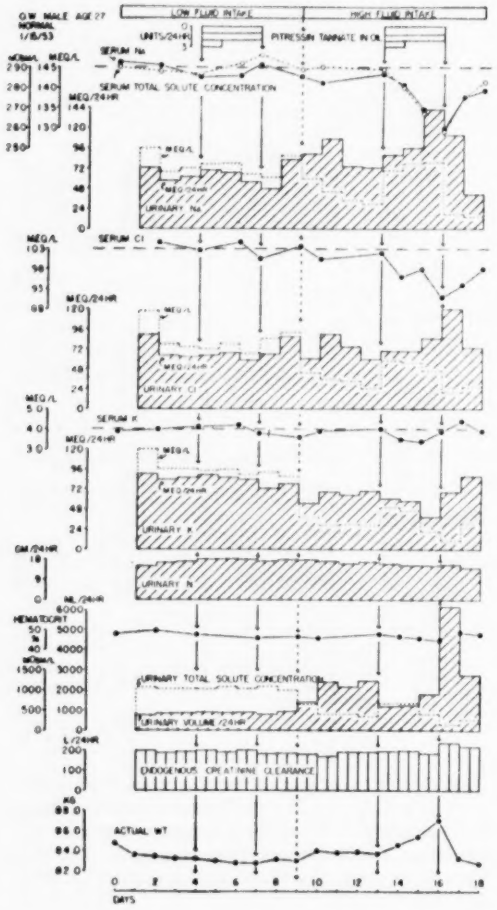

FIG. 4. THE EFFECTS OF PITRESSIN ON SERUM ELECTROLYTE AND TOTAL SOLUTE CONCENTRATIONS, URINARY ELECTROLYTE AND NITROGEN EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, HEMATOCRIT, ENDOGENOUS CREATININE CLEARANCE, AND BODY WEIGHT IN A NORMAL MAN ON LOW AND HIGH FLUID INTAKES

Fluid restriction prevented all of the effects of pitressin (weight gain, serum dilution, sodium and chloride loss) observed during the high fluid intake. Sodium intake was 78 mEq. per day by analysis.

[7] Although the sodium and chloride excretion had not reached "base-line" control values by the time the third course of pitressin was started, the effects on salt-loss are unequivocal.

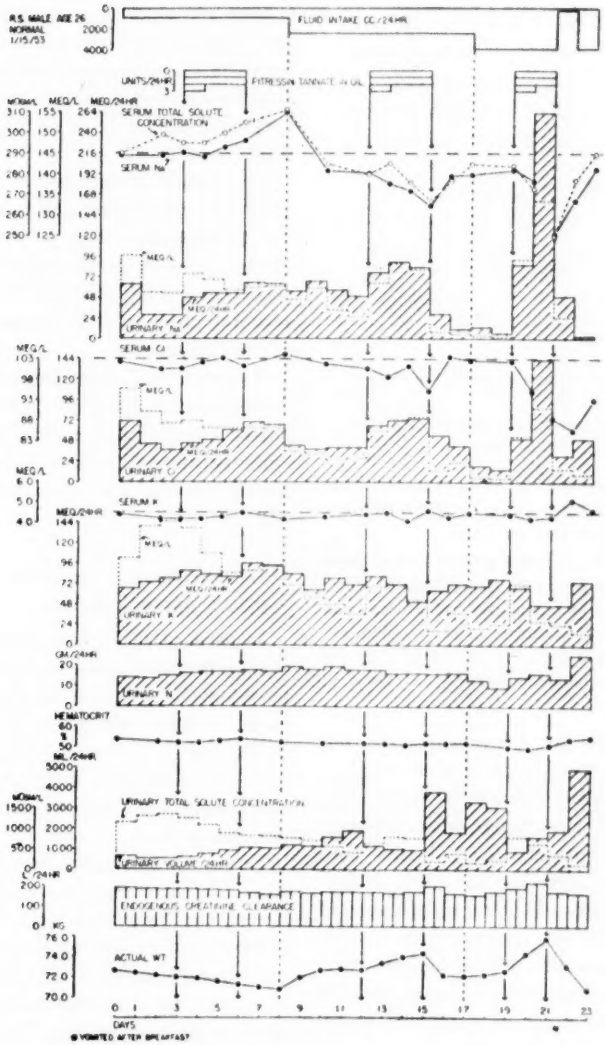

FIG. 5. THE EFFECTS OF PITRESSIN ON SERUM ELECTROLYTE AND TOTAL SOLUTE CONCENTRATIONS, URINARY ELECTROLYTE AND NITROGEN EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, HEMATOCRIT, ENDOGENOUS CREATININE CLEARANCE, AND BODY WEIGHT IN A NORMAL MAN DURING THREE DIFFERENT LEVELS OF FLUID INTAKE

During dehydration there was no loss of sodium in excess of intake, which by analysis was 60 mEq. per day. With a moderate and a large increase in water intake, pitressin produced corresponding degrees of fluid retention and sodium and chloride loss. The severity of dehydration on the low fluid intake in this subject was indicated by the high serum sodium and total solute concentrations and the weight loss. The progressive fall in urine total solute concentration and increase in urine volume, in spite of this dehydration and in spite of pitressin is unexplained and was not observed in other studies.

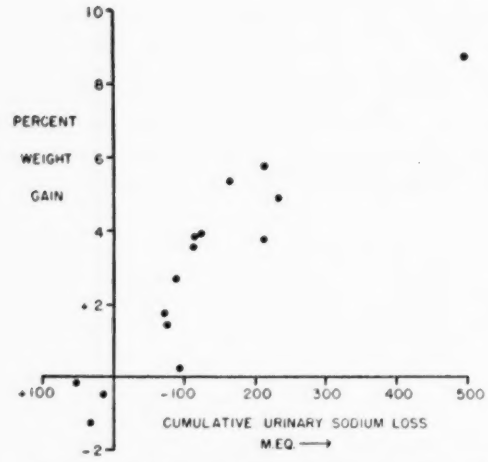

FIG. 6. THE RELATIONSHIP BETWEEN PITRESSIN-INDUCED WATER RETENTION (PER CENT INCREASE IN BODY WEIGHT) AND CUMULATIVE SODIUM LOSS

The data include all the pitressin periods except those during ACTH and very low sodium intake, both of which prevented sodium loss. The cumulative sodium loss was calculated as that in excess of control urinary sodium excretion and per cent weight gain was calculated from the maximum weight increase achieved during pitressin.

7.0 units of pitressin had previously been administered over three days in most of the studies. Therefore, this dose was administered to one subject, O.W., all at one time in five injection sites, in order to produce hormone levels higher than any that could have occurred in the other studies. Antidiuresis was accompanied by a weight gain of only 0.5 per cent (0.4 Kg.), and no increase in sodium or chloride excretion. This indicates that the large sodium and chloride losses from pitressin in these experiments cannot be explained as effects of excessive doses of pitressin.

C. *Prevention of the effects of pitressin on electrolyte excretion by ACTH*

In two studies adrenocorticotropic hormone was administered concomitantly with pitressin to see whether the sodium and chloride excretion could be prevented by stimulation of the adrenal cortex. Figure 7 shows the results in one subject; the other subject responded in similar fashion. Pitressin alone produced the expected fluid retention, weight gain, serum dilution and diuresis of sodium and chloride. A potent corticotropin,[8] 0.5 mg. administered subcutaneously every six hours for five days, completely abolished the sodium and chloride diuresis during pitressin; in fact, sodium excretion was markedly inhibited until ACTH was stopped.

The maximum increase in body weight was 2.3 kilograms when pitressin alone was administered but 3.1 kilograms when pitressin and ACTH were given. In spite of the larger fluid retention that occurred during the ACTH administration the serum dilution was less because of the concomitant sodium retention.

A very low sodium intake, 20 mEq. per day, during another study on this subject likewise prevented the expected sodium loss during pitressin administration. Such a low sodium intake is a potent stimulus for renal conservation of sodium (10).

D. *Hemodynamic and renal changes during pitressin administration*

In none of the subjects was there a significant change in blood pressure during administration of pitressin. Expansion of plasma volume subsequent to water retention was shown in subjects O. W. and R. S. The peak increase of plasma volume (as calculated from changes in hematocrit and hemoglobin) was 8 per cent in O. W. and 13 per cent in R. S.

In the three studies in which renal clearances were measured they were found to be increased during the fluid retention. In subject R. S. the clearance of endogenous creatinine rose from a mean control level of 171 ± 11 liters per day to a maximum of 217 liters during the second day of the last pitressin period. In O. W. the clearance of endogenous creatinine rose from a mean control level of 189 ± 9 liters per day to a maximum of 226 liters on the day after the second pitressin period.

In subject M. K. H. the inulin clearance rose from control values of 105, 102, and 85 ml. per minute per 1.73 m^2 to 124 ml. per minute per 1.73 m^2 during the last day of the first pitressin period in Figure 7. The simultaneous clearances of para-aminohippurate showed no significant changes.

[8] Generously supplied by Dr. E. B. Astwood.

E. *The effects of pitressin on excretion of other electrolytes and nitrogen*

In the two studies in which calcium and phosphorus balances were measured there occurred an increase in urinary calcium excretion similar to that shown in Figure 3. Changes in phosphorus excretion were equivocal. No constant pattern of potassium excretion was noted. Urinary nitrogen was not significantly affected by pitressin administration (Figures 4 and 5).

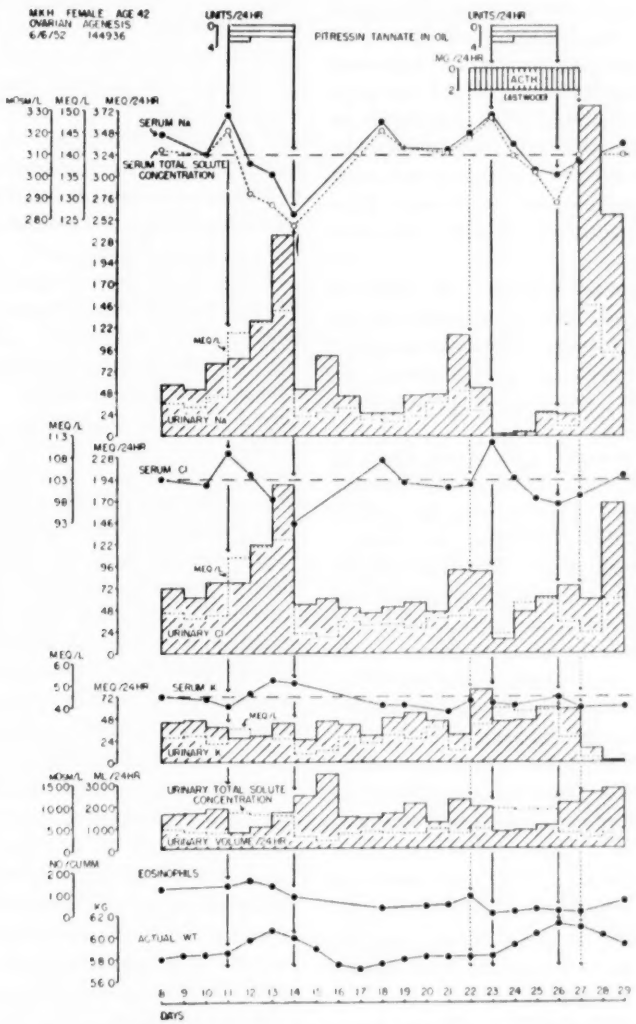

FIG. 7. THE EFFECTS OF PITRESSIN, ALONE AND WITH ACTH, ON SERUM ELECTROLYTE AND TOTAL SOLUTE CONCENTRATIONS, URINARY ELECTROLYTE EXCRETION, URINARY VOLUME AND TOTAL SOLUTE CONCENTRATION, CIRCULATING EOSINOPHILS, AND BODY WEIGHT IN A SUBJECT WITH OVARIAN AGENESIS

The natriuresis and chloruresis induced by pitressin were completely abolished by ACTH. The serum dilution was less with ACTH together with pitressin than with pitressin alone, although the weight gain was greater.

DISCUSSION

The confusion that exists regarding the effects of pitressin on renal excretion of electrolytes is probably the result of a diversity of experimental conditions. Species' differences, variations in dose, and the use of different extracts may have contributed to the conflicting findings. The present study indicates that the state of hydration of the experimental subject may be an important variable. Thus, dehydration prevented the large excretion of sodium and chloride produced by pitressin in hydrated subjects. This finding is in accord with the observation that endogenous antidiuretic hormone, released by dehydration (11–13), does not cause natriuresis or chloruresis (14, 15).

That pitressin does not exert a direct effect on electrolyte excretion in man is suggested by the results of the present study. The increased electrolyte excretion associated with pitressin appeared to be secondary to water retention and was prevented by dehydration.

The possibility, however, of a direct effect of pitressin on renal excretion of sodium and chloride has not been excluded. Mechanisms for sodium retention may come into play during dehydration and obscure a pitressin effect. Increased adrenal cortical activity was capable of inhibiting the natriuresis and chloruresis, and it is possible that dehydration stimulates the adrenal cortex.

Further evidence that pitressin has no direct electrolyte effects was seen in the dissociation between its rapid antidiuretic action and the late increase in electrolyte excretion which occurred in the hydrated subjects. That this late effect was not simply the result of an accumulation of pitressin activity in excess of physiological amounts is indicated by the following: *1*) no such effect was seen from the same pitressin dosage during dehydration;[9] *2*) when the total dosage was administered at one time to a hydrated subject there were no electrolyte effects.

The effects of a given quantity of water retention on sodium diuresis could be prevented by adrenal stimulation or salt restriction.[10] This was demonstrated by studying exactly comparable pitressin periods at the same level of fluid intake, in the same subject, "on" and "off" ACTH, and on low and high salt intakes. Thus it was apparent that this degree of water retention had not served to release endogenous sodium-conserving mechanisms.

In these experiments, small doses of pitressin were given over periods of several days, and it is possible that acute effects were obscured. However, several recent studies in which physiological amounts of posterior pituitary extract were given to human subjects in acute experiments reveal no effect on sodium and chloride excretion (1, 16–20).

Water retention with dilution of extracellular fluid, when marked, was accompanied by symptoms of water intoxication. These were progressive mental confusion, fatigue, headache, and nausea leading to vomiting. Muscle cramps occurred in only one instance. No essential differences were observed in the response of normal subjects and those with endocrine disorders, including adrenal cortical insufficiency. In two subjects, cortisone neither diminished the antidiuretic response to pitressin nor ameliorated the symptoms of water intoxication. Adrenocorticotropic hormone, on the other hand (Figure 7) did not diminish the antidiuretic response to pitressin, but did ameliorate the symptoms of water intoxication, presumably by causing sodium retention, and hence less serum and extracellular fluid dilution.

Low serum sodium concentrations have been considered to be a cause of decreased renal function. Serum sodium levels as low as 120 mEq. per L. were found in this study in the absence of any evidence of diminished renal function. Inulin, para-aminohippurate, and creatinine clearances, as well as renal concentrating ability, were unimpaired. This would suggest that the cause of reduced renal function in other low sodium states is not the low serum sodium concentration per se, but rather some associated abnormality, such as a

[9] Theoretically at least, during dehydration, administered pitressin is superimposed upon endogenous antidiuretic hormone, so that the total quantity of circulating hormone is greater than that obtaining when pitressin is administered to the hydrated subject.

[10] Since an increase of glomerular filtration rate was found to accompany water retention, it is likely that water retention of a sufficient magnitude could produce sodium loss in spite of a maximal stimulus to tubular reabsorption of sodium.

reduced plasma volume or cardiac output commonly found in these disorders.

It has been postulated that edema may result from an excess of endogenous antidiuretic hormone activity (21, 22). The assumption is made that primary water retention will cause sodium retention and the resultant isotonic over-expansion of extracellular fluid volume which characterizes the edematous state. The present studies, though of short duration, suggest that this sequence of events does not occur. Pitressin had no effect on the dehydrated subject; when the fluid intake was increased water retention occurred but sodium loss rather than retention ensued. It appears that antidiuretic hormone activity itself, in the absence of simultaneous and independent mechanisms for sodium retention, does not result in edema. No edema was noted in our subjects; indeed it is unlikely that pure water retention could cause significant clinical edema without lethal water intoxication.

Some increase in glomerular filtration apparently occurred during the water retention and natriuresis. However, the demonstration that ACTH prevented such sodium and chloride loss indicates that tubular reabsorptive capacity for sodium and chloride had not been exceeded. The finding of large excretions of sodium and chloride at a time when their concentrations in the serum were falling, clearly indicates that serum concentrations of sodium and chloride were not effective stimuli for their renal conservation. It is thought that this renal loss of sodium and chloride in the presence of obligatory retention of water is a homeostatic response to over-expansion of fluid volume.

It is of interest that Stewart and Rourke (23) produced an increased excretion of sodium by acutely over-loading a normal subject with water to the point of water intoxication. No posterior pituitary extract was administered to their subject. Strauss, Davis, Rosenbaum, and Rossmeisl (24) have increased renal sodium excretion by expansion of extracellular fluid volume with hypotonic saline solutions. These results appear to be manifestations of the same phenomenon: increased sodium excretion in response to over-expansion of body fluid volume.

SUMMARY AND CONCLUSIONS

The effects of a long-acting posterior pituitary extract on electrolyte and water excretion were studied in man. Observations were made during twenty 2 to 4 day periods of pitressin (1 unit per 12 hours) in nine subjects maintained on constant diets and fluid intakes.

It was found that pitressin produced in normally hydrated subjects the following: *1*) prompt water retention and resulting weight gain; *2*) serum dilution; and *3*) a marked increase in urinary sodium and chloride excretion manifest on the second or third day of pitressin administration.

Cessation of pitressin administration was followed by: *1*) a large water diuresis with fall in body weight; *2*) return of serum concentration to control levels; and *3*) a decrease in urinary sodium and chloride excretion to levels below the control.

All the above changes were prevented by restriction of fluid intake during pitressin administration. This strongly suggests that the increased excretion of sodium and chloride was the result of water retention and not a direct effect of pitressin.

Some increase in glomerular filtration rate apparently occurred during the water retention and natriuresis. Evidence that the tubular reabsorptive capacity for sodium and chloride had not been exceeded, however, was indicated by the prevention of such electrolyte loss by ACTH or by a low sodium diet.

The renal loss of sodium and chloride resulting from obligatory retention of water is interpreted as a homeostatic response to over-expansion of fluid volume.

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EFFECTS OF CORTICOTROPIN ON BODY WATER AND ELECTROLYTES IN PATIENTS WITH RHEUMATIC DISEASE [1]

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The therapeutic response to pituitary adrenocorticotropic hormone is often accompanied by profound changes in water, solid, and electrolyte distribution. Metabolic balance studies in patients receiving corticotropin (1) have demonstrated marked initial retention of sodium and chloride and subsequently increased excretion of these ions while potassium balance remained negative. Bartter and his co-workers (2), studying patients with panhypopituitarism who were receiving corticotropin, observed that retention of sodium was greater than that of chloride, and concluded that sodium entered cells when corticotropin was given, and left the cells during the recovery periods. There was a large but transient loss of potassium within 24 hours after the beginning of treatment and a corresponding retention of potassium at the beginning of the post-corticotropin period. A transient increase in the inulin space in patients treated with corticotropin and cortisone, and maintained on rigid salt restriction, has been reported by Levitt and Bader (3). Since the patients studied by these authors were on a markedly restricted salt intake and remained in salt balance, without gain in weight, it was concluded that the observed increase in the extracellular fluid volume was due to a shift of salt and water from the intracellular compartment. Recently, Ziff, Simson, and Bunim (4) reported variation in the manner of water retention by different individuals during corticotropin administration. This was predominantly intracellular in some cases and extracellular in others, as determined from the simultaneous measurement of antipyrine and bromide spaces. The present study is designed to quantitate further some aspects of the changes in body water and electrolyte metabolism which may be encountered during this induced state of adrenalcortical hyperfunction.

METHODS

Extracellular fluid volume was measured by the calibrated infusion technique (5) using sucrose. Total body water was determined by the antipyrine [3] method (6). Total exchangeable sodium and potassium (7) and chloride (8) were measured by dilution techniques employing radiosodium (Na^{24}), radiopotassium (K^{42}) and bromide. Extracellular and non-extracellular [4] electrolyte and total body solids were calculated as described in previous papers. Extracellular sodium and potassium were calculated as the products of extra-cellular fluid volume and plasma concentration. Corrections for plasma water ($W = 0.92$) and the Donnan factor ($K = 1.02$) have been made in the calculations of extracellular chloride.

When bromide studies (9) were repeated at weekly intervals in the measurement of body chloride, significant concentrations of bromide were present in control plasmas. Correction for the fall in the control level of bromide during the 24-hour equilibration period, the magnitude of which varied from two to eight per cent, was made as described by Ziff, Simson, and Bunim (4).

The patients were given a standard hospital low salt

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[3] Antipyrine was supplied in sterile ampules by Eli Lilly & Co.

[4] By non-extracellular electrolyte, we mean the part of total body electrolyte which is not contained in the extracellular fluid volume as measured by the sucrose volume of distribution. Thus, non-extracellular electrolyte includes all intracellular electrolytes as well as the portion of total exchangeable electrolyte which may not literally be within the limits of cell membranes (bone sodium, for example).

diet which, by dietetic analysis, had an average daily composition of carbohydrate 230 Gm., protein 55 Gm., fat 55 Gm., potassium 2.8 Gm., sodium 0.40 Gm. and chloride 0.66 Gm. This diet was supplemented by 4 Gm. of sodium chloride which was presented to the patient in a weighed salt shaker. All patients were on the diet and salt supplement for at least five days prior to the control study. The patients consumed the full diet and salt supplement throughout the period of observation. Corticotropin was administered intramuscularly every six hours in the dosages indicated in the tables.[5]

The patients were as follows:

Initials	Age	Sex	Diagnosis
T. T.	15	Male	Rheumatoid Spondylitis
R. F.	14	Male	Rheumatic Fever
H. C.	17	Female	Rheumatic Fever
W. C.	42	Female	Rheumatoid Arthritis

Clinical summaries are appended.

RESULTS

The results (Tables I–IV) in the four patients studied are summarized as follows. Three of the patients (T. T., R. F., W. C.) gained weight (range 1.9 to 8.5 Kg.) while one (H. C.) had lost 4 Kg. at the end of 22 days of corticotropin therapy. The values for total body water and total body solids indicate the nature of these weight changes. Of the three patients who gained weight, T. T. and R. F. (Tables I and II) present a similar pattern. The progressive increase in total body water reflects a continuing positive water balance which is most marked during the first week of treatment . This fluid is retained as cell water, as the values for extracellular and intracellular water indicate. As a result of initial decrease in extracellular water, however, cellular hydration is increased by an amount greater than the volume of water retained. Thus the positive water balance is associated with a shift of extracellular water into the cells. Restoration of extracellular fluid volume was complete in T. T. (Table I) after 43 days of treatment and partial in R. F. (Table II) after 34 days of treatment. Both patients displayed a marked decrease of total body solids during the first five-day period, and this value continued to decrease as shown by tests performed on the thirteenth day in R. F. and the nineteenth day in T. T. Side effects of corticotropin administration such as moon face and acne were prominent when the final measurements were made in these patients, at which time the mass of total body solid had returned to values greater than those measured in the control study. This requires the assumption of a positive solid balance with deposition of either body fat or protein late in the course of corticotropin treatment.

In W. C. (Table III), the gain in weight was related to a different sequence of events. No significant change in total body water occurred during twelve days of treatment although a transient shift of 2.4 liters of cell water to the extracellular fluid was observed on the fifth day. The increase in body weight during this period was due to an increase in body solid. After two weeks, the patient began to gain weight rapidly, concurrently developing signs of marked extracellular fluid volume expansion, that is, peripheral edema, ascites, pulmonary congestion, and elevated venous pres-

[5] Corticotropin (pork ACTH) was supplied by Armour & Co. Dosages are expressed in milligrams equivalent to Armour standard LA-1-A.

TABLE I

Rheumatoid spondylitis

T. T., 15 years, ACTH dosage = 100 mg./day

Day of ACTH administration	Control	5	19	43
Weight (Kg.)	28.3	30.0	31.3	36.8
Total body water (liters)	16.1	19.3	21.9	23.2
Extracellular water (liters)	8.5	7.2	7.5	8.5
Intracellular water (liters)	7.6	12.1	14.4	14.7
Total body solids (Kg.)	12.2	10.7	9.4	13.6
Total body sodium (mEq.)	1,450.	1,480.	1,520.	1,790.
Plasma sodium concentration (mEq./L.)	140.	139.	136.	144.
Extracellular sodium (mEq.)	1,190.	1,000.	1,020.	1,220.
Non-extracellular sodium (mEq.)	260.	480.	500.	570.
Total body chloride (mEq.)	1,210.	1,160.		1,480.
Plasma chloride concentration (mEq./L.)	106.	105.		108.
Extracellular chloride (mEq.)	1,110.	840.		1,020.
Non-extracellular chloride (mEq.)	100.	320.		460.

TABLE II

Acute rheumatic fever

R. F., 14 years, ACTH dosage = 100 mg./day

Day of ACTH administration	Control	5	13	34
Weight (Kg.)	53.9	56.0	55.5	60.5
Total body water (liters)	34.7	38.2	39.5	40.5
Extracellular water (liters)	13.3	12.4	10.4	11.5
Intracellular water (liters)	21.4	25.8	29.1	29.0
Total body solids (Kg.)	19.2	17.8	16.0	20.0
Total body sodium (mEq.)	2,200.		2,340.	
Plasma sodium concentration (mEq./L.)	134.	133.	135.	140.
Extracellular sodium (mEq.)	1,780.		1,410.	
Non-extracellular sodium (mEq.)	420.		930.	

sure, which did not respond to salt restriction, diuretics or digitalis therapy. After the study on the nineteenth day, corticotropin was discontinued. The increase of 7.5 liters in extracellular fluid from day 12 to 19 was effected by a positive water balance of 4.3 liters and a shift to the extracellular fluid of 3.2 liters of cell water. Total body solids continued to increase during this interval.

Although H. C. (Table IV) lost weight, the alterations induced by corticotropin in this patient are of a similar pattern to those initially noted in W. C. Total body water decreased progressively during treatment, indicating a negative water balance. This water loss was met exclusively from the intracellular water since the data show that extracellular fluid volume did not fall below the control value at any time during treatment. A transient shift of cell water to the extracellular fluid was noted in this patient (H. C., Table IV, Day 15). In contrast to T. T. and R. F. who had a marked decrease in body solids during the initial period on corticotropin, H. C. and W. C. registered increases in total solids indicating positive fat or protein balance.

Serial measurements of total body sodium and its distribution were performed in three patients, T. T., R. F. and H. C. (Tables I, II, and IV). Corticotropin induced sodium retention with an increase in total body sodium in each patient. The sodium distribution measurements indicate that all or the greater part of this retained sodium became part of the non-extracellular electrolyte. A comparison of the control value for non-extracellular sodium with the first measurement after institution of corticotropin therapy showed an increase of 180 per cent in T. T., 220 per cent in R. F. and 130 per cent in H. C.

Changes in the amount and distribution of body chloride were observed in three patients. Two patients, H. C. and T. T. (Tables I, IV) showed a pattern for chloride similar to that described for sodium. There was retention of chloride, and increase in non-extracellular chloride after 15 and 43 days of treatment, respectively. In W. C., the

TABLE III

Rheumatoid arthritis

W. C., 42 years, ACTH dosage = 100 mg./day

Day of ACTH administration	Control	5	12	19
Weight (Kg.)	50.2	50.2	53.4	58.6
Total body water (liters)	29.9	29.5	29.0	33.3*
Extracellular water (liters)	10.4	12.8	10.7	18.2
Intracellular water (liters)	19.5	16.7	18.3	15.1
Total body solids (Kg.)	20.3	20.7	24.4	25.3
Total body chloride (mEq.)	1,730.	1,870.	1,910.	2,530.
Plasma chloride concentration (mEq./L.)	108.	109.	104.	101.
Extracellular chloride (mEq.)	1,250.	1,550.	1,240.	2,070.
Non-extracellular chloride (mEq.)	480.	320.	670.	460.

* Determined by D_2O Method. Analyses were carried out by Mr. Irving Sucher of Columbia University on the mass spectrometer. Blood samples were collected till 12 hours after injection for determination of equilibrium concentration of D_2O.

TABLE IV

Rheumatic fever and rheumatic heart disease
H. C., 17 years, ACTH dosage = 100 mg./day

Day of ACTH administration	Control	6	15	27
Weight (Kg.)	52.7	51.0	50.9	48.7
Total body water (liters)	32.4	30.3	30.1	27.5
Extracellular water (liters)	10.2	10.4	11.5	10.3
Intracellular water (liters)	22.2	19.9	18.6	17.2
Total body solids (Kg.)	20.3	20.7	20.8	21.2
Total body sodium (mEq.)	1,840.	2,010.	2,120.	1,990.
Plasma sodium concentration (mEq./L.)	140.	139.	142.	138.
Extracellular sodium (mEq.)	1,430.	1,450.	1,630.	1,420
Non-extracellular sodium (mEq.)	410.	560.	490.	570.
Non-extracellular sodium concen. (mEq./L.)	18.	28.	26.	33.
Total body chloride (mEq.)	1,590.		1,870.	
Plasma chloride concentration (mEq./L.)	108.		102.	
Extracellular chloride (mEq.)	1,220.		1,300.	
Non-extracellular chloride (mEq.)	370.		570.	

early transient extracellular shift of water and the later development of increased extracellular fluid volume account for the observed changes in body chloride.

DISCUSSION

The present studies of some aspects of the changes in body composition induced by corticotropin, as measured by dilution techniques, are in substantial agreement with the findings of Bartter and his co-workers (2) who applied metabolic balance methods to the same problem. The latter workers demonstrated the positive sodium and chloride balances which may occur and inferred that the sodium is retained in large measure as intracellular sodium. Our data corroborate this positive balance and demonstrate that non-extracellular sodium and chloride may be greatly increased in some patients undergoing treatment.

The data relating to body solids and body water distribution indicate a variable response to the administration of corticotropin. W. C. and H. C. revealed a decrease in intracellular water volume which was maintained throughout the period of observation (despite the development of extracellular fluid retention in W. C. after twelve days of treatment). Each of these patients also demonstrated an increase in extracellular fluid volume at a time relatively early in treatment. Both patients showed progressive increase in total body solids during the course of therapy.

The body composition changes in T. T. and R. F. were in sharp contrast to the changes described in H. C. and W. C. The progressive increase in total body water in T. T. and R. F. reflected an increase in cell water. The extracellular fluid volume which had been initially decreased as a result of intracellular water shift was partially or completely restored at the time of the last measurement. An initial large decrease in total body solids occurred and continued for at least the first two weeks of treatment. This effect had been completely reversed in both patients at the time of the last measurement (43 and 34 days) when total solids were found to be greater than the control value.

These studies fail to demonstrate a single pattern of response to corticotropin. There probably are numerous biologic variables, known and unknown, which determine the clinical and metabolic pattern of response in a particular patient. The functional state of the adrenal cortex at the time corticotropin is administered may be one factor of significance in the observed variability of effects among individuals. Animal studies by Deane, Shaw and Greep (10) and Bacchus (11) have demonstrated by cytologic methods that an increase in the dietary sodium-potassium ratio produced evidence of decreased activity in the glomerular zone of the cortex, while lowering of the ratio produced changes indicative of increased activity in this area. Thus the previous level of sodium intake is probably one element influencing the effect of corticotropin. It has been suggested that androgenic hormone is derived from the reticular zone (12) and corticosteroids from the fascicular zone of the cortex (13). Such precise division may not be correct, but variables may

exist which affect these areas of the cortex differentially, and which obviously cannot be properly controlled before they have been defined. The observation that the levels of dietary fat and carbohydrate may significantly modify the clinical and metabolic effects of corticotropin (14) is another indication that the intensity of the adrenocorticotropic stimulus is not the only determinant of the degree and nature of adrenal cortical activity. The nature and degree of illness as well as the impact of corticotropin therapy on the illness itself, in contrast to the effect of the hormone on water distribution, may also influence the response.

SUMMARY

1. Four patients with rheumatic diseases were treated with corticotropin for periods ranging between nineteen and forty-three days.

2. Two patients retained water intracellularly during the first week of treatment, and simultaneously showed a shift of extracellular water into the cells. The decrease in extracellular fluid volume was subsequently restored on further treatment. In two patients, intracellular water was decreased throughout therapy and a transient shift of cell water to the extracellular fluid was noted early in treatment. One of these patients developed a marked increase in extracellular fluid volume after two weeks of corticotropin.

3. Total body solids decreased and then rose in two patients, and increased progressively in two patients.

4. Sodium retention was induced in three patients as indicated by a rise in total body sodium. All or most of this retained sodium became part of the non-extracellular electrolyte.

5. In two of three patients, there was retention of chloride mainly as non-extracellular chloride. In the third patient, changes in total body chloride were consistent with changes in extracellular fluid volume.

6. Under the conditions of these studies, markedly different patterns of response to corticotropin have been observed.

CASE SUMMARIES

1. *Rheumatoid spondylitis:* T. T., a fifteen-year-old Chinese boy, had a history of rheumatoid arthritis since seven years of age. There had been three exacerbations of the disease during the intervening period. On admission to the hospital, he was afebrile. He appeared thin but well developed. The erythrocyte sedimentation rate was 94 mm. per hour (Westergren method), but other routine laboratory tests and electrocardiogram were within normal limits. There was tenderness of the knees and ankles as well as limitation of hyperextension of the hips. X-rays revealed generalized changes of rheumatoid arthritis and ankylosing spondylitis. He was treated with corticotropin in a dosage of 100 mg. daily for eight weeks with lessening of joint pain and inflammation but no decrease in deformities. In response to corticotropin therapy, the erythrocyte sedimentation rate fell gradually to 9 mm. per hour in 45 days. Two eosinophile counts were 65 and 38 per mm^3., respectively, before treatment; during treatment numerous counts ranged between 12 and 37 per mm^3.

2. *Rheumatic fever:* R. F., a fourteen-year-old white boy, was admitted to the hospital with a history of fever and polyarthritis of two weeks' duration. The temperature was 103.4° F. on admission. He was moderately well nourished and well developed. There was no evidence of carditis on physical examination. The knees, ankles, and feet were swollen and tender. Erythrocyte sedimentation rate was 92 mm. per hour, C-reactive protein 4+ and antistreptolysin titer 300 and 500. X-ray of the chest was within normal limits. Aspirin therapy, which was continued for one month, produced an immediate fall in temperature and erythrocyte sedimentation rate with relief of symptoms. Following withdrawal of aspirin, however, there was an exacerbation of symptoms. An early aortic diastolic murmur was heard for the first time. Corticotropin was then administered in a dosage of 100 mg. daily for 50 days. There was no further polyarthritis or fever, and the erythrocyte sedimentation rate fell from 60 to 20 mm. per hour in the first 3 weeks. The eosinophile count fell from 100 to 42 per mm^3. on the second day. When corticotropin treatment was halted after 50 days, there was no relapse. The aortic diastolic murmur was not heard after 31 days of corticotropin and did not reappear when corticotropin was discontinued. X-rays of the chest showed the heart to be of normal size and shape at the time of discharge.

3. *Rheumatic fever:* H. C., a seventeen-year-old white girl, had previous episodes of rheumatic fever at six and nine. Two months prior to admission, the patient developed migratory arthralgias and continued low grade fever. There were no symptoms of diminished cardiac reserve. On admission, the temperature was 100.4° F. and pulse 120 per minute. Blood pressure was 120/95 mm. Hg. The heart was not enlarged, but a diastolic rumbling murmur was heard at the apex. Except for slight warmth in the knees, there were no other findings of significance on physical examination. Routine laboratory tests and electrocardiogram were within normal limits. Erythrocyte sedimentation rate was 9 mm. per hour. Corticotropin was injected in a dosage of 100 mg. daily. The sedimentation rate remained within normal limits and the eosinophile count fell from 87 to 9 per mm^3. After 28

days of treatment the patient had a psychotic episode and corticotropin was discontinued two days later. There was no evidence of diminished cardiac reserve during the period of treatment.

4. *Rheumatoid arthritis:* W. C., a forty-two-year-old, normotensive, white woman, had a history of increasing pain and stiffness in numerous joints over a period of 13 years. She was afebrile on admission and during the pretreatment period. Significant findings on physical examination were confined to the joints. There was slight to moderate restriction of movement of the shoulders, ankles, elbows, and wrists and small joints of the hand with moderate pain on passive motion. Routine laboratory tests and electrocardiogram were normal. The erythrocyte sedimentation rate ranged between 33 and 45 mm. before corticotropin treatment. Corticotropin was administered in a dosage of 100 mg. daily. This resulted in relief of joint pain and decrease in stiffness. The sedimentation rate fell to 25 mm. per hour in eight days and the eosinophile count from 87 to 7 per mm^3. The patient gained 5.2 Kg. between the twelfth and nineteenth day of therapy. She became dyspneic and orthopneic. Rales were heard at both bases and there was an increase in heart size and hilar congestion on X-ray. Venous pressure rose to 200 mm. of saline. Following discontinuation of corticotropin therapy, there was a large diuresis with loss of edema and 9.1 Kg. of weight in two days. The lungs cleared and dyspnea disappeared. There was no relapse of the arthritis during the following eight-month period.

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SOME STUDIES OF POSTERIOR PITUITARY AND ADRENAL CORTICAL INTERRELATIONSHIPS IN PATIENTS WITH AND WITHOUT CIRRHOSIS OF THE LIVER[1,2]

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It is well recognized that both the adrenal cortical and posterior pituitary hormones play an important part in the regulation of salt and water metabolism (1). Antagonistic relations between these two hormones have been postulated (2). It has also been shown that in the human a reciprocal relationship exists between the antidiuretic activity of serum and the corticosteroid of urine (3). During diuresis, the antidiuretic activity in serum is low and the corticosteroid excretion in urine is high, whereas, during water retention, the converse situation is found.

Increased amounts of antidiuretic material were recovered from patients with active cirrhosis of the liver by Ralli, Robson, Clarke, and Hoagland (4). In the experimental animal, the liver has been found to be the most effective site of inactivation of the posterior pituitary hormone (5, 6, 7). It was decided, therefore, to study the effects in patients with liver disease of artificially elevating the levels of circulating antidiuretic material by the administration of posterior pituitary extract.

This report is concerned with: a) the changes in metabolism of water and sodium resulting from repeated administration of pitressin tannate in oil, and b) the relationship between plasma sodium and urinary excretion of corticosteroid. The response to exogenous adrenal cortical steroids given concomitantly with posterior pituitary extract was also studied.

METHODS

The studies of the response to pitressin tannate injected daily have been carried out on five patients with Laennec's cirrhosis and on five "control" patients recovering from various diseases, but without evidence of Laennec's cirrhosis. In addition, one patient (Case 11), a confirmed alcoholic with history of phosphorus poisoning as a child, but with no laboratory evidence of cirrhosis, was studied. He was under treatment for erythromelalgia following frost-bite. Further study, reported under Case 11 in the Appendix, provided evidence of some degree of adrenal insufficiency. For these reasons the patient could be considered neither as a control patient nor a patient with liver disease.

All of the cirrhotics had had ascites, but at the time of the studies only one patient was in positive water balance. The subjects were maintained on a controlled diet of known sodium content and five Gm. of salt in solution was given in addition each day. Fluid intake was maintained at a constant level for each patient, usually 2,500 cc. each day. Measurements of weight, urine volume, and urine sodium and potassium were made daily. Frequent determinations of plasma sodium and potassium were obtained by means of the flame photometer. Urinary corticosteroid was measured by a method which has been previously described (8). The normal values range from .200 to .750 mg. of corticosteroid per 24 hours. Pitressin tannate was administered intramuscularly in oil in a dose of 5 units once daily for approximately the first week of the experiment. In several cases, when no effect upon water or electrolyte excretion was observed, the dose was increased to ten or more units daily in two divided doses. When the administration of pitressin tannate had caused significant water retention and depletion of plasma sodium levels, adrenal cortical extract, *i.e.*, lipo-adrenal cortex (Upjohn) intramuscularly, aqueous cortical extract (Upjohn) intravenously, was given while the pitressin tannate injections were continued. The course in each patient is reported separately in the Appendix.

RESULTS

The Effect of Continued Administration of Pitressin Tannate upon Water and Sodium Balance

Injection of pitressin tannate resulted in weight gain from water retention in all patients, but of varying degree.

[1] This paper was read in part before the American Society for Clinical Investigation in May, 1951.

[2] This work was in part supported by grants from the Ciba Company and the Upjohn Company. The pitressin tannate used was supplied through the courtesy of Dr. D. A. McGinty of Parke, Davis & Company. The adrenal cortical extract and lipo-adrenal cortex was supplied by Dr. H. F. Hailman of the Upjohn Company.

For the purpose of comparison, Cases 1–5 (see Appendix) are reported together as control patients and Cases 6–10 are reported as cirrhotic patients. Case 11 is reported separately.

Figure 1 records the weight changes of the cirrhotic and control patients up through the seventh day of the study (fourth day of pitressin administration). Both groups received 5 units of pitression tannate in oil intramuscularly daily. After the seventh day of the study, the sensitivity responses were not entirely comparable since pitressin dosage was increased in several cases. Some subjects were given adrenal cortical hormone after this date and were, therefore, not included in the graph after such administration.

By the fourth day of the administration of five units of pitressin tannate daily intramuscularly, the cirrhotic group had an average weight gain of 6.4 lbs., a maximum of 13 lbs. Control subjects gained an average of 2.9 lbs. The maximum was 7 lbs. of fluid retained. There is considerable spread in the weight gains recorded for individual subjects. The one cirrhotic who did not gain weight failed to do so because of severe vomiting.

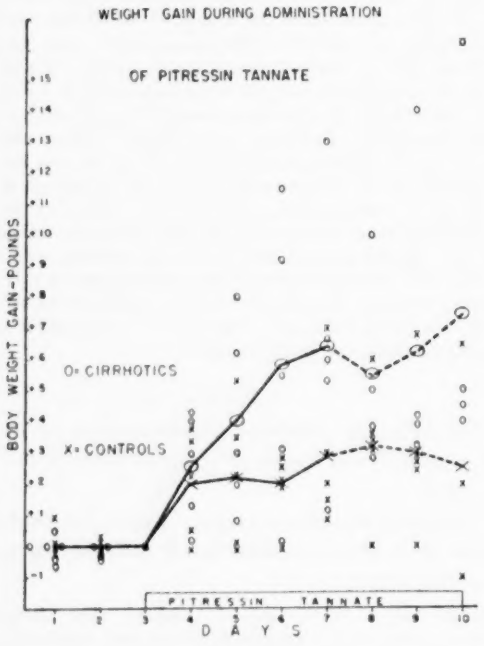

FIG. 1.

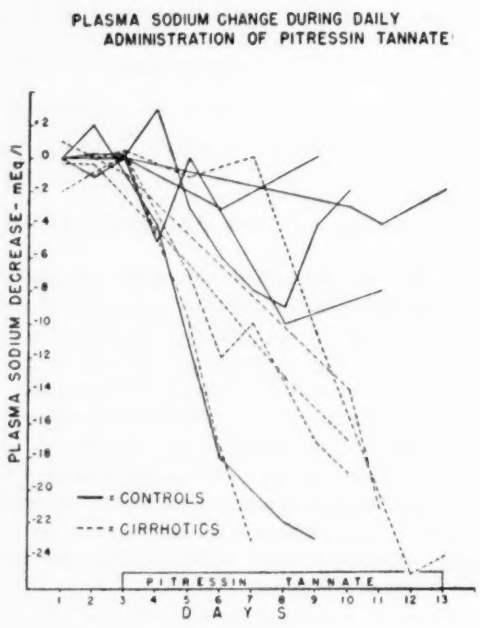

FIG. 2.

The remaining cirrhotics all gained more than five pounds and the amount of fluid lost as vomitus by the fifth cirrhotic was considerably more than five pounds. One of the control subjects had a weight gain comparable to the average of the cirrhotics. None of the remaining control patients gained more than three pounds of weight. For these reasons, it is felt that although the groups are too small to permit positive proof of statistical significance of the differences in the groups, the data suggest a definite trend for the cirrhotic group to gain more weight than the control group.

Continued administration of pitressin tannate also produced a decrease in plasma sodium in both groups. Figure 2 compares the degree of response in the two groups during this part of the study. The cirrhotic patients responded with a dramatic drop in plasma sodium levels ranging between 17 and 25 mEq. per liter. Only one control patient (Case 5) evidenced a comparable plasma sodium decrease. The remaining four control subjects responded with only a moderate hyponatremia of 2 to 10 mEq. per liter. The plasma sodium levels could not be well correlated with water retention and weight gain in all in-

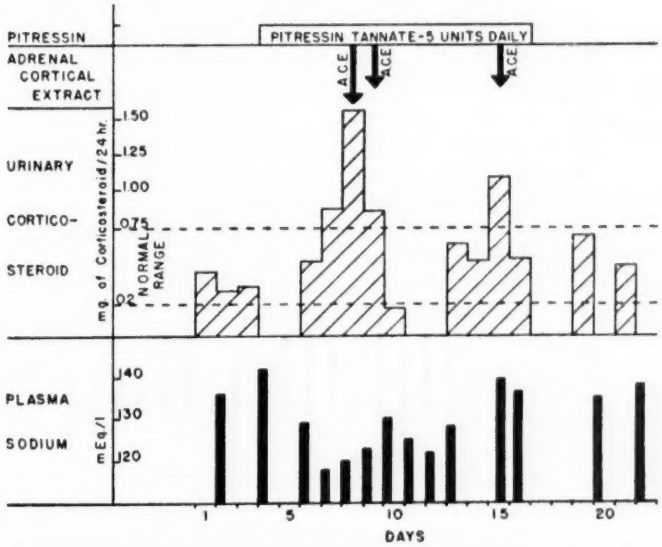

FIG. 3 (CASE 11). ALTERATIONS IN PLASMA SODIUM AND URINARY CORTICOSTEROID LEVELS OCCURRING DURING ADMINISTRATION OF PITRESSIN TANNATE AND ADRENAL CORTICAL EXTRACT

dividuals. In the control group, Case 5, who responded after six days of 5 units of pitressin daily with a precipitous drop in the plasma sodium level from 142 to 117 mEq. per liter, retained only 2.8 lbs. of fluid. Meanwhile, the patient who gained the most weight in the control group (Case 2), showed only a moderate hyponatremia. Similarly, in the cirrhotic group, Case 10, whose plasma sodium level fell from 133 to 108 mEq. per liter, retained only 2 lbs. of fluid. This patient, however, lost considerable fluid through vomiting. In most of the patients with cirrhosis an antidiuresis with increasing edema and ascites was associated with a progressive drop in plasma sodium levels.

Case 11 is reported separately for reasons described under Methods. Figures 3 and 4 record the response of this patient following pitressin tannate administration. Within four days, the plasma sodium level fell from 139 to 120 mEq. per liter and body weight increased from 109 to 116 lbs.

The Effect of Continued Administration of Pitressin Tannate on Urinary Corticosteroid

The excretion of corticosteroid has been studied during daily pitressin tannate administration in seven subjects; three with diagnoses of Laennec's cirrhosis, one with chronic alcoholism, and three with no evidence of liver disease. All determinations of corticosteroid excretion in the urine were within the normal range during the control period except in two individuals with cirrhosis whose corticosteroid excretions were .95 mg. per 24 hrs. and .81 mg. per 24 hrs., respectively.

No appreciable increase in urinary corticosteroid was observed in any subject until a significant decrease in plasma sodium had occurred. During the time that the plasma sodium was maintained at a low level with the continued administration of pitressin tannate, the urinary corticosteroid usually remained above the control levels. Figure 5 illustrates that during the continued administration of pitressin tannate to a normal subject who had little change in plasma sodium, there was no increase in urinary corticosteroids. Figure 6 illustrates that when pitressin tannate produced a striking depression of serum sodium in the cirrhotic, there was a concomitant increase in corticosteroid.

Chromatographic fractionation of corticosteroids excreted by one of these patients, Case 10, has

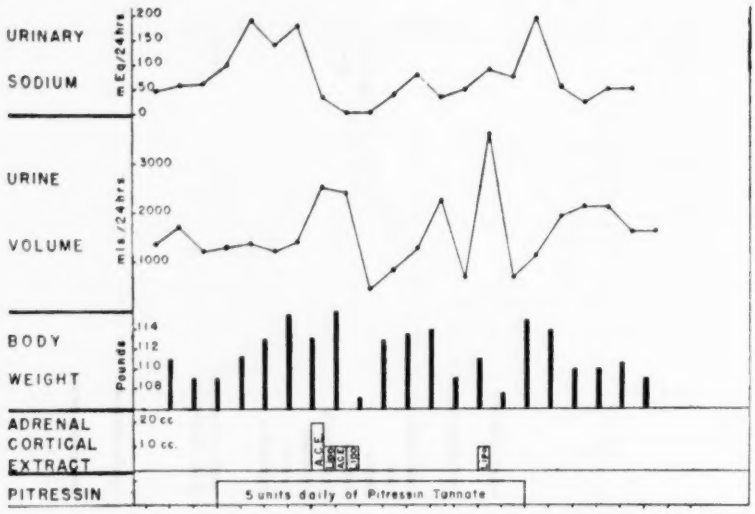

FIG. 4 (CASE 11). ALTERATIONS IN URINARY SODIUM EXCRETION, URINE VOLUME, AND BODY WEIGHT OCCURRING DURING ADMINISTRATION OF PITRESSIN TANNATE AND ADRENAL CORTICAL EXTRACT

demonstrated an alpha-ketol which moves more rapidly than desoxycorticosterone, and which forms formaldehyde upon oxidation with periodic acid. Based on the rate of flow, it seems probable that this material contains three oxygens. No further identification has as yet been possible.

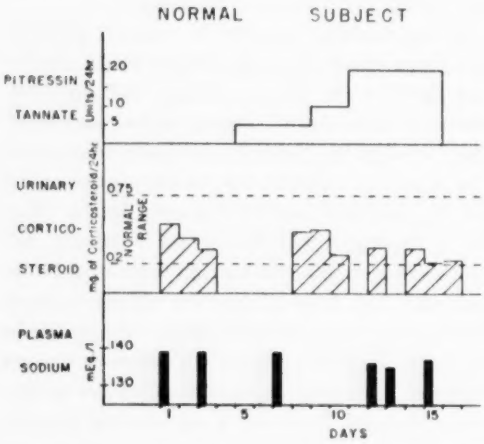

FIG. 5. EFFECT OF CONTINUED ADMINISTRATION OF PITRESSIN TANNATE ON PLASMA SODIUM AND URINARY CORTICOSTEROID—NORMAL SUBJECT

The Effects of Exogenous Adrenal Steroids on Water and Electrolyte Disturbances Caused by the Continued Administration of Posterior Pituitary Extract

Two patients from the control group, five patients with cirrhosis and Case 11 received adrenal cortical extract during the course of pitressin tannate administration. Adrenal cortical extract appeared to promote salt retention in two controls (Cases 2 and 3) when the pitressin dosage was maintained at 5 units per day, but no effect was apparent in Case 3 after pitressin dosage had been increased to 15 units per day.

Adrenal cortical extract was administered for a total of 17 experimental days in the cirrhotic group. It is worth noting that the urine volume and sodium excretion varied considerably from day to day, making it difficult to assess the effects of adrenal cortical extract. Furthermore, when antidiuresis was very pronounced, the urine sodium content was markedly reduced secondary to oliguria (see Case 9). On only two of the 17 experimental days (Day 20, Case 6; Day 17, Case 10) did a sodium retention result which might be ascribed to the effects of adrenal cortical extract. Adrenal cortical extract failed to promote

an obvious diuresis in either the control or the cirrhotic patients. In Case 7, however, the degree of water retention and weight gain was less after adrenal cortical extract, and in Case 10 a definite weight loss occurred concomitantly.

The hyponatremia resulting from pitressin tannate administration was improved in only one patient (Case 10).

In Case 11, pitressin tannate administration promoted an antidiuresis and natriuresis, both of which were reversed with adrenal cortical extract. Antidiuresis returned as pitressin was continued and was again reversed with adrenal cortical extract, although there was little immediate effect upon sodium excretion. The hyponatremia resulting from pitressin tannate tended to be corrected also.

DISCUSSION

These experiments have raised the following points for discussion: The tolerance of patients with liver disease to pitressin; the causes of the resulting hyponatremia; the effects of pitressin administration upon adrenal cortical function; and the reversal of the effects of pitressin by adrenal cortical extract.

White, Rubin, and Leiter (9), and Nelson and Welt (10) have found that when relatively small doses of pitressin are given in an acute experiment, no difference in effect is found, and they concluded that the patient with cirrhosis can inactivate physiological doses of pitressin as well as the normal. Our preliminary experiments with small amounts of aqueous pitressin yielded the same result.

It seems probable that extrahepatic sites of pitressin inactivation are capable of removing the antidiuretic activity present in small amounts of pitressin given either intravenously or subcutaneously. Fairly large doses of pitressin are required to demonstrate a defect in hepatic inactivation. Eversole, Birnie, and Gaunt (6) found it necessary to give a dose of 40 milliunits of pitressin to 200 Gm. rats in order to demonstrate differences in effectiveness of the intrasplenic or subcutaneous routes. This dose is approximately fifteen times the amount of pitressin required to produce a state of antidiuresis. Heller (11) has found that rabbit blood is capable of inactivating several milliunits of pitressin per cc. of blood and believes that this inactivation actually represents a loose combination with protein. Birnie, Jenkins,

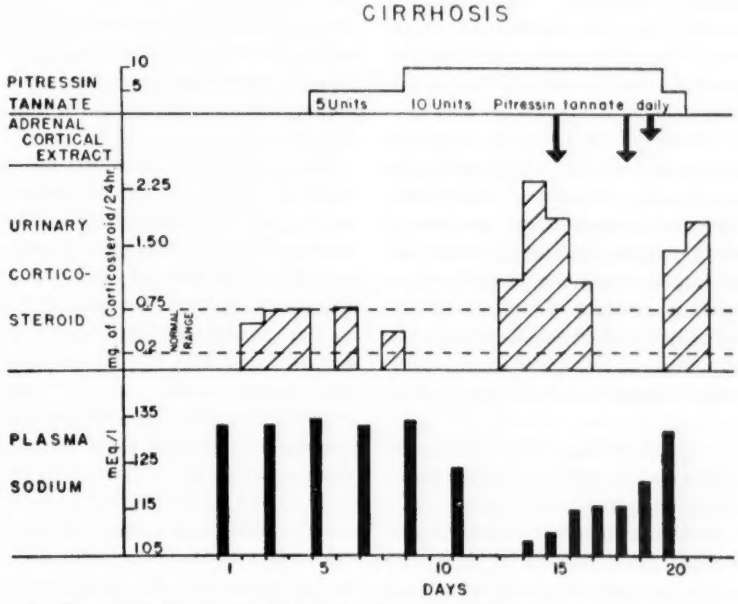

FIG. 6. EFFECT OF CONTINUED ADMINISTRATION OF PITRESSIN TANNATE ON PLASMA SODIUM AND URINARY CORTICOSTEROID—PATIENT WITH CIRRHOSIS

Eversole, and Gaunt (12) have found that rat serum is capable of destroying the antidiuretic activity in pitressin and a few preliminary studies in our laboratory indicate that human blood has the same property.

The data of these experiments suggest that by taking advantage of a possible accumulative effect, differences in rate of inactivation of pitressin by patients with and without liver disease can be shown. Although the liver is the most active single tissue in pitressin inactivation, it is only one of many mechanisms which remove antidiuretic activity from blood. Therefore, even if the hepatic inactivating mechanisms are strikingly less efficient than normal, the total overall ability of the body to inactivate pitressin may not be very greatly decreased and the defect is made apparent only when large amounts of pitressin are administered over a period of several days.

Animal experiments by Loewy and Lloyd (13) have compared the effectiveness of pitressin tannate given to normal rats and to rats with fatty livers produced by diet low in protein and high in fat. The fatty livers were capable of inactivating *in vitro* only one-third of the antidiuretic activity of an amount of pitressin which normal liver could completely destroy. These findings agree with those of Birnie, Blackmore, and Heller (14), who reported that a low protein diet greatly decreases the pitressin inactivating ability of the mouse liver *in vitro*.

The relatively minor differences in the response between the cirrhotic and the normal group in this study and the complete absence of differences between cirrhotics and normals when pitressin is given intravenously suggest that hepatic inactivation of posterior pituitary hormone probably plays relatively little part in removal of antidiuretic activity from blood under normal physiological conditions and that failure of this process may very well have only minor importance in the production of ascites.

Bartter (15), in discussing an experiment carried out in collaboration with Leaf, has pointed out that the normal individual may be quite sensitive to pitressin tannate with rather striking water retention. Our experiences are certainly in agreement. There is no question that certain normal subjects have considerable antidiuresis as a result of pitressin tannate administration, but the degree of antidiuresis is usually not as marked as in the cirrhotic and frequently, relatively little effect is apparent.

The natriuretic and antidiuretic effects of exogenous pitressin could be minimized presumably by various hormonal and physiologic mechanisms within the body. If this were so, abrupt withdrawal of exogenous pitressin would be expected to lead to sudden and profound diuresis with rapid return of the plasma sodium level to initial levels. This occurred in eight of the ten patients followed. The effects of withdrawal of pitressin were far more obvious than the slow accumulative effects of pitressin administration. The pattern of changes resulting from pitressin administration suggested by these studies are supported, therefore, by the dramatic reversal of effects on discontinuing pitressin tannate.

The effect of prolonged administration of pitressin tannate on the plasma sodium could be mediated in three possible ways. A natriuresis frequently occurred following administration of pitressin tannate in oil. Other investigators, who have studied the excretion of sodium following the administration of aqueous extract of pitressin have been unable to demonstrate a natriuresis in the acute experiment. By the methods used in this study, however, an increased loss of sodium in the urine occurred in four patients, (Cases 5, 6, 7, 10). Equally significant was the acute retention of sodium when pitressin tannate was abruptly withdrawn.

The second way in which the plasma sodium might be depressed is by dilution with retained water. In some patients, this could account for most or all of the decrease in plasma sodium, since as much as ten liters of fluid were retained. It is difficult to account for the decrease in plasma sodium in other patients, however, by either an increased sodium excretion or by simple dilution with retained water. A third mechanism must be considered, that is, a redistribution of sodium from an extracellular to an intracellular position. The suggestion that this may occur has also been made by White, Rubin, and Leiter (9). Pitressin causes a shift of sodium into tissue from plasma in guinea pigs (16). Keutmann (17) has been able to demonstrate an increased intracellular sodium concentration in several patients with edema occurring as a result of heart failure. Whether such

an intracellular shift of sodium in the series here presented might have occurred in response to pitressin administration, or whether it might have been mediated through an increased amount of circulating adrenal steroids containing three oxygens, is as yet undetermined. The latter possibility must be considered since suggestive evidence has been presented that an increased amount of a three oxygen containing steroid was present in the urine of one patient, and it has been shown that desoxycorticosterone is capable of causing a shift of extracellular sodium into the cell (18). If the alpha-ketol demonstrated in the urine of one patient in this series had physiological activity similar to desoxycorticosterone, it might have been responsible for such a shift. However, the only time that the three oxygen containing steroid was found in these patients was when the plasma sodium level was already at a low level, so that it now seems more likely that the hyponatremia might very well be the stimulus which caused the appearance of the three oxygen containing steroids. A shift of sodium to the intracellular position would seem to be a direct pitressin action since this shift has been reported to occur (9) during the acute administration of small amounts of pitressin.

The lack of direct effect of pitressin tannate administration on adrenal cortical steroid excretion is apparent. Only when the plasma sodium is depressed to a level which is associated with signs of the low salt syndrome could an increased amount of steroid be discovered in the urine. The failure to find an increased amount of steroid does not mean, of course, that the gland itself is not producing more, since it is easily possible that the increased output of the gland might be balanced by increased utilization with the result that no additional hormone was available for wasting in the urine. However, the studies of Nagareda and Gaunt (19) have shown that pitressin in physiological amounts does not cause a change in adrenal ascorbic acid. Studies on the urinary corticosteroid and serum antidiuretic levels during water diuresis have shown that a reciprocity exists and that the antidiuretic activity decreases and the corticosteroid level increases during water diuresis. Since this reciprocity is not a direct one, the hypothesis has been made that a response to a common stimulus (hypotonicity) decreases secretion of posterior lobe hormone and increases secretion of adrenal cortical steroids.

The increased level of adrenal cortical steroids in diuresis, and the known opposing effects of adrenal steroids and pitressin, constituted the rationale for administration of adrenal cortical extract concomitantly with pitressin tannate. Only in Case 11 in whom there was some clinical evidence of adrenal insufficiency, did adrenal cortical extract produce an obvious diuresis with retention of sodium. In several of the remaining cases, an apparent retention of sodium occurred on some occasions, but without obvious diuresis. The failure to produce this effect more frequently may be the result of an inadequate dosage. No attempts to reproduce these experiments using cortisone or 17-hydroxycortisone have been made.

SUMMARY

1. Pitressin tannate in oil was given by intramuscular injection daily to five patients with cirrhosis of the liver and to five "control" patients. One patient, a chronic alcoholic with evidence of poor adrenal function, was also studied.

2. Administration of pitressin tannate in oil can produce hyponatremia and antidiuresis in both the control and the cirrhotic patients. These effects are greater in the cirrhotic, and frequently lead to a stage of progressive hyponatremia and edema.

3. The opposite effect, profound diuresis and return of plasma sodium towards normal, occurs promptly when exogenous pitressin is withdrawn.

4. Pitressin administration lowers plasma sodium levels by at least two mechanisms: Antidiuresis with dilution of extracellular sodium by retained water; and a direct natriuresis. A third mechanism must be considered: A shift of sodium from the extracellular to the intracellular space.

5. Urinary excretion of corticosteroid increases when plasma sodium concentration falls to low levels, but does not increase as a result of pitressin administration *per se*.

6. Adrenal cortical extract produced an obvious diuresis and retention of sodium in only one patient who had developed water retention and hyponatremia as a result of pitressin tannate administration. In several other patients an apparent retention of sodium occurred on occasion without obvious diuresis.

APPENDIX

37 year-old white male. Diagnosis - myeloradiculopathy, achlorhydria. No history of liver disease or alcoholism.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			139	1680	168	67	0.54	139	4.3	116	Showed no evidence of edema at any time.
2			139	1260	86	53	0.42				
3			139	1750	70	66	0.32	139	4.2		
4	5u		139	1060	94	60		139	4.1		
5	5u		139.5	1255	113	72					
6	5u		139	1525	130	126					
7	5u		139	1285	100	48	0.47				
8	10u		140.5	2050	142	84	0.50				
9	10u		139	1520	103	60	0.30				
10	20u		139	1495	81	69					
11	20u		138	1000	139	75	0.36	136	4.7		
12	20u		138	2070	164	76		135			
13	20u		138	1000	108	81	0.35				
14	20u		140	885	130	61	0.21	137	3.8		
15			144	1215	144	65	0.25				
16			143	5200	81	114					
17			136.5								

CASE I—M. C.

36 year-old white male. Diagnosis - migrating thrombophlebitis. Denies alcoholism or history of liver disease. Liver not palpable. Liver function tests normal.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			153.8	2200	132	43	0.54	143	5.1		Actual salt intake unknown.
2			155	1195	95	42	0.36				
3			154.3	2295	124	40		142	4.3		
4	5u		154	780	102	57		143	3.7	90	No apparent edema at any time.
5	5u		157.8	2720	98	59		138	3.7		
6	5u		157.5	790	42	45		143	4.4		
7	5u		156.5	960	136	56	0.73				
8	5u		161	2270	119	56	0.59				
9	5u		160	1560	123	53	0.70	133	5.2		
10	5u		160.8	1920	101	50					
11	5u		160.5	1605	149	72					
12	5u	10L* - 10A	162.8	2100	85	32		135	4.7		
13	5u		161.3	2580	193	56		130	4.5		
14			161	3790	13	52					
15			155.5					142	4.2		

*L = Lipo-adrenal cortex

A = Aqueous adrenal cortex

CASE II—J. W.

22 year-old white male. Recovered from mild hepatitis with jaundice. Denies alcoholism. Liver not enlarged. Cephalin flocculation - trace. Bromsulphthalein retention (5 mg./kg.) 0% at 60 mins. Albumin 5.1 gm.%, globulin 2.5 gm.%. Eosinophil response to adrenalin: basal 122/mm³, 4 hrs 57/mm³. Urinary 17-ketosteroid excretion 15 mg./24 hrs. Diet high in potassium due to protinal and fresh fruit supplement.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			164.5	2500	119	118				106	No edema or ascites
2			165	1920	119	93					
3			164	1300	33	100					
4			164	1540	22	121					
5	5u		164	1200	44	113					
6	5u		166.3	2060	180	113		140	4.4		
7	5u		166.3	2320	122	102					
8	5u		167	960	60	73		137	4.5		
9	5u		167	1920	65	120					
10	5u		167	2680	60	144					
11	5u	10L - 10A*	167	1320	27	103		140	4.7		
12	5u	15L - 23A	168	1880	59	106		136	4.4		
13	5u		166	2080	92	112		135	4.0		
14	10u		169.5	1380	152	135		135	4.0		
15	10u		171.5	2320	58	157					
16	10u		170	1380	66	130		133	3.8		
17	15u		170	1160	64	159					
18	15u		174.3	1460	166	108		129	4.1		
19	15u	10L - 20A	177	1100	111	119		127	4.2		
20	15u	15L - 20A	181	1140	90	84		125	4.1		
21			178	2190	159	117					
22			175.5	6840	68	68		128	4.3		
23			166	1880	6	85					
24			167.8					138	3.9		
25								145	3.4		

*L= Lipo-adrenal cortex
A= Aqueous adrenal cortex

CASE III—S. S.

33 year-old Negro male. Recovered from lobar pneumonia. Denies alcoholism. No evidence of liver disease. House diet. Fluids ad lib. Urine not collected.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			150					142	5.2	Approx. 190	No edema at any time during study.
2	5u		150					142	5.1		
3	5u		150					145	5.0		
4	5u		150					139	4.5		
5	5u		152					136	5.3		
6	5u		152					134	4.5		
7	5u		153.5					133	4.4		
8	5u		152.5					138			
9			152					140	3.8		
10			151.5						4.3		
11			152					141			

CASE IV—J. S.

56 year-old white woman. Diagnosis - neurasthenia. EEG suggestive of epilepsy. History of repeated trauma to head. Denies alcoholism or liver disease. Bromsulphathalein retention (5 mg./Kg.) 0% at 45 mins. Cephalin flocculation 2+ repeat trace. Albumin 4.3 gm.%, globulin 2.1 gm.%. Eosinophil response to adrenalin: basal 480/mm³, 4 hrs. 133/mm³. Urinary 17-ketosteroid excretion 3.8 mg./24 hrs.

Day	Pitressin Tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			129	1732	125	61	0.42	140	4.6	116	No edema, ascites
2			129.8	2130	167	74	0.52				
3			129.8	2558	123	82		142	4.3		Abd. circ. 34½".
4	5 u		130	1440	147	94					
5	5 u		133.5	1700	282	75		136	4.9		
6	5 u		135.3	1535	242	74					Vomited 1200 cc.
7	5 u		132	1070	48	48	0.78	122	4.0		Abd. circ. 37", ankle
8	5 u		131	1355	94	56	0.58	120	4.5		edema 1+.
9	5 u		133.5	2350	265	66		118	5.7		
10			132.8	2122	75	43		117	5.4		Abd. circ. 38", ankle edema cleared
11			127	3810	5.6	32		120	5.4		
12			123.8	1805	7.6	55		133	5.4		Abd. circ. 34"
13			126					135	3.9		

CASE V—G. H.

45 year-old white male. Diagnosis - Laennec's cirrhosis. History of prolonged alcoholism. Liver and spleen enlarged. Bromsulphathalein retention (5 mg./kg.), 19% at 45 mins. Cephalin flocculation 4+. Albumin 3.3 gm.%, globulin 4.3 gm.%. Eosinophil response to adrenalin: basal 230/mm³, 4 hrs. 124/mm³.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			158	2720	126	61		136	3.8	109	No evidence of edema or ascites
2			158	2000	93	65					
3	5u		158	1620	138	71		138	3.6		
4	5u		159.3	1360	109	57					
5	5u		160	1420	135	50		131	4.3		
6	5u		161	1675	171	55		126	3.8		
7	5u		163.3	2100	192	60		128	4.3		
8	5u		161	1780	107	63					
9	10u		162	1720	96	52		121	4.3		
10	10u	20L-20A*	162.5	1920	61	68		119	3.9		2+ ankle
11	10u		163.8	2480	67	62		119			edema.
12	10u		163.5	2600	77	50		123	3.8		Ankle edema
13			162	4240	12	56		126			clearing.
14			158.3	2900	37	80		135	3.8		
15			158.5	2560	56	60					
16			158.5	2400	78	62		136	4.1		
17	5u		158.5	1910	137	63		136	3.9		
18	5u		159.5	1755	156	63					
19	5u		160.3	2160	216	63		127	3.5		
20	5u	15L-20A	160.5	1465	39	75		125	4.1		
21	5u	10L-20A	161	2140	93	74		128	3.8		
22	5u		161	1700	94	46		126	3.6		
23	5u		160	2218	144	57		128	3.4		
24			162.5					124	3.6		Transferred from hospital.

*L=Lipo-adrenal cortex
A=Aqueous adrenal cortex

CASE VI—C. C.

42 year-old white female. Diagnosis - Laennec's cirrhosis. History of alcoholism. Liver and spleen enlarged. Bromsulphathalein retention (5 mg./kg.) 28% in 45 mins., cephalin flocculation 1+. Albumin 3.6 gm.%, globulin 2.0 gm.%. Liver biopsy showed marked fatty metamorphosis. Eosinophil response to ACTH: basal 294/mm^3, 4 hrs. 118/mm^3.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			176	2440	51	47	0.81			116	
2			176	1545	72	26					No ankle edema or ascites.
3			175.3	1860	65	23					
4			175.5	2140	65	37	0.80				Abd. circ. 43½".
5	5u		176.5	465	92	43		139	4.2		
6	5u		180	547	93	30	0.38				Abd. Circ. 43½"
7	5u		184	600	113	40	0.55	129	3.8		
8	5u		187.5	465	95	25		121	4.1		Abd. circ. 46"
9	5u	20L-20A*	189	1170	175	77	1.02	116	4.3		2+ edema
10	5u	20L-20A	188.5	1040	92	55		113	4.1		Abd. circ. 44"
11	5u		190.8	1615	167	40		113			
12	5u		191.5	1805	160	45	0.97	113	4.2		3+ ankle edema
13	5u		191.3	1075	95	39	0.68	112	4.7		Abd. circ. 48".
14			193.5	5425	83	54	1.30	110	5.2		Face puffy
15			182.3	4690	76	52		129	4.4		Edema cleared
16			177.8								Abd. circ. 42"

*L = Lipo-adrenal cortex
A = Aqueous adrenal cortex

CASE VII—N. K.

39 year old white female. Diagnosis - Laennec's cirrhosis. History of chronic alcoholism. Liver enlarged, tender. Two previous paracenteses. Bromsulphathalein retention (5 mg./kg) 14% at 45 mins. Cephalin flocculation 2+. Albumin 3.0 gm.%, globulin 3.6 gm.%. Eosinophil response to adrenalin: basal 744/mm^3, 4 hrs. 600/mm^3. Urinary 17-ketosteroid excretion 1.3 mg./24 hrs.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24hrs.	Remarks
1			111	1930	29	36	0.58	139	4.0	109	Ascites minimal
2			110.3	1420	21	26	0.62	139	3.2		No ankle, sacral edema
3			110.5	690	20	15					
4	5 u		110.8	355	10	10			4.5		
5	5 u		113.8	275	7	20					
6	5 u		117	430	7	22		132	3.5		
7	5 u		120	1940	8	27					
8	5 u		117.5	880	20	33	1.21				Increasing ascites
9	5 u		120.8	410	5	23		126	4.8		Ankle edema 2+
10	5 u		124.8	345	3	15					Ankle and sacral edema
11	5 u	10L*	127	410	1.3	14		122	4.4		Marked ascites
12	5 u	10L - 20A*	129	315	2.3	24		121	3.6		(Hemorrhage from bowel. Transfusions 500 cc. blood)
13	5 u	10L - 37A	130	590	4.4	21		120	3.7		
14	5 u		133.3** 125	410	0.7	18		120	4.0		Abd. paracentesis.
15			126.8	3470	3	27					
16			119.5	3280	0.8	25		128	3.7		
17			116	1860	0.8	15		129	3.7		
18			114.8	900	0.3	7					
19			114.5	2310	43	59					
20			115	1120	3	16		131	2.5		

*L=Lipo-adrenal cortex
A=Aqueous adrenal cortex

**Wts. before and after abdominal paracentesis

CASE VIII—M. P.

76 year old white woman. Diagnosis-Laennec's cirrhosis. Denies history of alcoholism or hepatitis. History of recent hematemesis from esophageal varices. Liver and spleen enlarged. Bromsulphathalein retention (5 mg./kg.) 20% at 45 mins. Cephalin flocculation 3+. Albumin 3.3 gm.%, globulin 3.3 gm.%. Fluid intake ad lib.

Day	Pituitrin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24hrs.	Remarks
1			124.8	1420	23	37				61	No evidence of edema
2				1235	27	51					
3				2240	35	31		133	4.4		
4			123.5	2285	53	63					
5				1918	61	41		132	4.3		
6	5 u		123	300	14	17					
7	5 u		127	1430	132	64					
8	5 u		126	390	6	20					
9	5 u		128.5	370	49	70					
10	5 u		129	2000	91	56					
11	5 u		128	2835	35	43					
12	10 u		126	1420	37	59					Apathetic
13	10 u		128	815	76	39		118	4.3	208	No edema
14	10 u	15L-20A*	131.5	1415	156	65		111	4.1	168	
15	10 u	17L-20A	132.8	495	18	21		112	4.0	199	Basilar rales
16			134.5	4520	121	52		117	3.9	156	Ankle and sacral edema
17			126.3	1520	40	21		132	3.6	156	Edema cleared
18			126.5	2825	215	35		133	4.4	190	
19			127	2860	170	37				156	
20			125.5	3440	162	55					
21	10 u		124.8	2255	159	53		134	4.0		
22	10 u		124.8	1860	110	51					
23	10 u		124	780	81	44		130	4.0		
24	10 u		126.3	2545	171	53		119	4.2		
25			125.8	4536	16	38					
26			121.5	2165	38	46		136	4.5		
27			122	1790	82	52					
28			123	1680	78	28					
29			123	2710	184	60					
30			122	2570	132	58					
31			123.5	1940	95	50					
32			123	2305	118	60					
33			123	2590	159	60					
34			123	2000	109	45		138	4.4		
35	10 u		123	2410	152	70					
36	10 u		123	1650	109	56					
37	10 u		125.3	950	112	40					
38	10 u	20L*	128.5	1340	190	45		114			Vomiting clear fluid
39	10 u	20L	130.3	1050	73	41		118	4.0		Severe precordial pain
40	10 u	20L-30A*	128	480	22	19		119	4.1		EKG negative
41	10 u	20L-30A	128.5	1310	124	38		113	3.4		
42	10 u		128.3	3060	67	18		118	3.2		
43	10 u		123.5	1180	49	23		129	3.9	Unknown	Gross hematemesis, shock received 200 cc. whole blood.
44	10 u			1215	11	21					
45				1455	117	12		140	3.5		

*L= Lipo-adrenal cortex

A= Aqueous adrenal cortex

CASE IX—E. M.

46 year old Negress. Diagnosis-Laennec's cirrhosis. Prolonged alcoholism. Liver much enlarged and tender. Bromsulphathalein retention (5 mg/kg.) 40% at 45 mins. Cephalin flocculation 1+. Albumin 3.6 gm.%, globulin 3.6 gm.%. Given 500 ml. 10% dextrose in water, 500 ml. 10% dextrose in saline, and 1000 ml. ammosol in 5% dextrose intravenously daily, plus oral feeding of special formula containing 30 mEq. Na+. Patient unable to take regular diet or fruit juice mixture. Periods of vomiting during the study.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			118	2135	95	41	0.58	133	3.9	108	No ankle edema
2				1988	82	46	0.73	133	4.0		or ascites
3			116	1812	34	41	0.95				Abd. circ. 37 in.
4	5 u			2615	93	51		134	3.4		
5	5 u		116	1695	95	38	0.71				
6	5 u		116.8	1430	59	27		133	3.8		
7	5 u		116	1460	85	39	0.47				Abd. circ. 38 ¾ in.
8	10 u		117	1350	120	40		134	3.9		
9	10 u		119.8	2465	165	41					
10	10 u		120	1940	158	47		124	3.6		
11	10 u		120	924	41	49					
12	10 u		120	1820	95	52	1.13				
13	10 u		118	1336	81	47	2.34	108	3.0		Abd. circ. 39 in.
14	10 u	20L*	118	2460	77	49	1.88	110	3.4		
15	10 u		115.5	2090	72	52	1.09	115	3.2		Diffuse joint tenderness
16	10 u		116	2430	83	32		116	2.8		Ankle edema, fingers puffy
17	10 u	20L-20A*	114	1410	15	27		116	3.0		Dull, apathetic
18	10 u		112	1335	22	23		121	2.7		
19	5 u	10L-20A	110	1730	51	23	1.50	132	2.9		Generalized convulsions
20				1230	54	18	1.86				Convulsions
21				1720	42	11					Abd. circ. 34 in.
22			111	908	14	6					Abd. circ. 35 ¾ in.

*L = Lipo-adrenal cortex
A = Aqueous adrenal cortex

CASE X—C. M.

59 year old white male. Diagnosis-erythromelalgia, phosphorus poisoning as a child. Chronic alcoholic. Liver not enlarged. Bromsulphathalein retention (5 mg./kg.) 0% at 45 mins. Albumin 4.9 gm.%, globulin 2.0 gm.%. Cephalin flocculation 1+. Urinary 17-ketosteroid exgretion 4 mg./24 hrs. Glucose tolerance curve flat. Eosinophil response to adrenalin: basal 88/mm³, 4 hrs. 105/mm³.

Day	Pitressin tannate units/day	Adrenal Cortical Extract cc./24 hrs.	Weight lbs.	Urine Vol. cc./24 hrs.	Urine Na mEq./24 hrs.	Urine K mEq./24 hrs.	Urinary Cortico-Steroid mg./24 hrs.	Plasma Na mEq./l.	Plasma K mEq./l.	Sodium Intake mEq./24 hrs.	Remarks
1			109	1355	49	25	0.43			90	1+ ankle edema with erythromelalgia
2			109	1690	62	20	0.32	139	3.9		
3			109	1210	66	25	0.33				
4	5 u		109	590	99	36		139	4.0		
5	5 u		111.8	1385	189	38					
6	5 u		113	1200	139	43	0.52	129	3.9		
7	5 u		115.5	1395	181	64	0.82	118	4.3		3+ leg edema
8	5 u	10L-20A*	116	2510	35	70	1.56	120	4.2		Face puffy
9	5 u	10L-20A	116	2300	5	21	0.86	123	4.5		
10	5 u		107	460	5	18	0.18	130	4.3		Edema cleared
11	5 u		113	850	39	26		125	4.3		
12	5 u		113.5	1250	81	33		122	5.9		Edema recurring
13	5 u		114.3	2250	37	25	0.62	128	4.6		
14	5 u		109	620	51	17	0.52				
15	5 u	10L	111	3575	91	33	1.09				
16	5 u		107.5	670	75	32	0.53	136	4.5		Edema cleared
17	5 u		115	1085	196	36					
18			114	1890	54	18					Edema recurring
19			110	2125	22	20	0.69				Edema cleared
20			110	2120	49	42		135	4.5		
21			110	1620	49	22	0.49				
22								138	4.4		

*L=Lipo-adrenal cortex
A=Aqueous adrenal cortex

CASE XI—J. M.

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NUTRITIVE ADEQUACY AND CLINICAL TOLERANCE OF MODIFIED HUMAN GLOBIN [1]

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The production of plasma and its fractions from whole blood has led to investigations of the usefulness of the red cell residues. Globin, the protein moiety of hemoglobin, can be obtained in quantity from the red blood cells remaining after the plasma has been removed. Modification [3] (1) of this protein renders it suitable for intravenous use. Previous studies suggest that this material may have osmotic properties useful in treating shock and may be a source of protein for parenteral administration (*2–7*). The present investigation was designed to elucidate further the usefulness of modified human globin with particular regard to its nutritive value and clinical tolerance in man.

MATERIALS AND METHODS

Clinical tolerance to intravenous infusions of modified human globin was observed in 20 convalescent patients, and the nutritive value of this protein was investigated by nitrogen balance studies in six male subjects. Four of these six subjects, M. M. age 50, J. R. age 35, F. O'D. age 60, and W. O. age 43, were essentially normal except for a moderate degree of undernutrition; one, J. B. age 43, had minimal cirrhosis of the liver proven by biopsy; and one, J. G. age 28, was a normal medical student. None of the patients, except J. B., had evidence of liver or renal disease. They were maintained on the Thorndike metabolic ward and offered diets providing constant intakes of protein and calories. Dietary nitrogen and calorie intakes were calculated from standard food tables (8, 9). Macro-Kjeldahl analysis of three separate lots of modified human globin yielded an average value for nitrogen content of 15.7 per cent. In these studies the conventional factor 6.25 was used to convert globin protein to nitrogen. The nitrogen content of the isoleucine used was calculated from the analytical data provided by the manufacturer. Analyses of the diets for nitrogen content were not done. In the case of the diets which were essentially devoid of protein, the errors introduced by the use of calculated values rather than analyzed values for nitrogen intake would be minimal. In the case of those subjects given diets containing moderate amounts of protein the nitrogen intake error might be greater, but since the subjects studied were maintained on constant diets before, during, and after globin administration this intake error would be constant and would not invalidate the observations concerned with the metabolism of globin. This intake error would however, detract from the precision of those studies designed to compare the nutritive adequacy of globin with that of dietary protein.

The patients were weighed daily under constant conditions. The 24-hour urine volumes were collected in 10 cc. of a 5:1 mixture of glacial acetic acid and toluol as preservative. Daily urinary nitrogen determinations were done by standard micro-Kjeldahl analysis. Urinary protein nitrogen was considered as the difference between total nitrogen and non-protein nitrogen after the removal of urine protein by precipitation with Folin-Wu reagent. Pooled stools, obtained during each control and experimental study period, were preserved with benzoic acid and refrigeration. The stool nitrogen content was determined by the macro-Kjeldahl technique.

The preparation of the modified human globin used in these investigations followed the method developed by Strumia and Sample (1) with suitable modifications instituted by the manufacturer (10). In the following studies globin was prepared for use as a 4 per cent solution in distilled water. This aqueous solution of globin produced hemolysis *in vitro*, so that now glucose or saline diluent is preferred.

Serum globin concentration was determined by the method of Kendall and Abell as described by Sample, Strumia, and Satta (11) and plasma and urine alpha amino nitrogen by that of Hamilton and Van Slyke (12).

RESULTS

I. *Nutritive adequacy.* Observations were made on the nutritive adequacy and the metabolism of modified human globin administered to six adult men.

[1] The expenses of this study were defrayed in part by a grant from Sharp & Dohme, Inc., West Point, Pennsylvania to Harvard University.

[2] Welch Fellow in Internal Medicine of the National Research Council.

[3] "Modified human globin" was furnished for this study by Dr. William Boger, Sharp & Dohme, Inc., Glenolden, Pennsylvania.

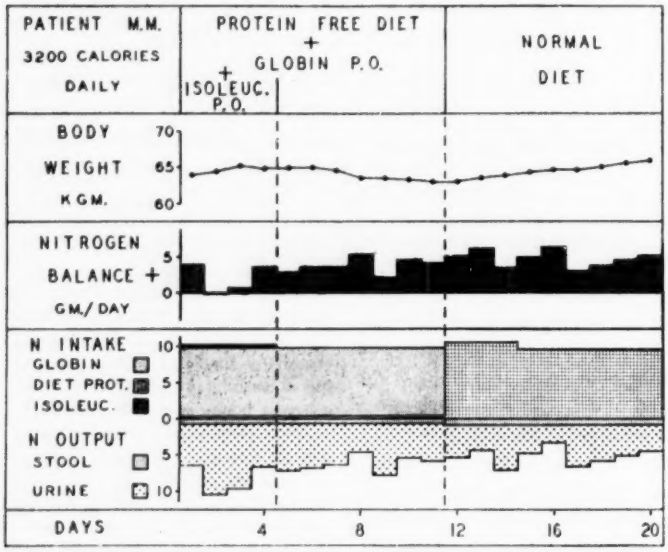

FIG. 1. MODIFIED HUMAN GLOBIN ADMINISTERED ORALLY AS A SOLE SOURCE OF PROTEIN WITH AND WITHOUT ISOLEUCINE

The nutritive adequacy of a protein is dependent in part upon its content of essential amino acids. Isoleucine is the essential amino acid present in globin in smallest concentration (Table I). The initial metabolic studies were designed to determine whether or not the low concentration of this amino acid limited the ability of globin to maintain nitrogen balance in adult man. Patient M. M. (Figure 1) was maintained on a constant diet providing 3,200 calories daily. This diet was essentially devoid of protein, and contained only 0.3 Gm. of nitrogen per day by macro-Kjeldahl analysis.[4] In addition to the basal diet, the patient received a multiple vitamin capsule daily.[5] During the first four days of study this patient ingested as a sole source of protein an amount of globin furnishing 9.6 Gm. of nitrogen daily. In addition, he received 1 Gm. dl-isoleucine[6] orally three times daily. During these four days nitrogen balance and body weight were maintained. On the fifth day isoleucine administration was stopped, but the globin was continued for the next seven

TABLE I

Analysis of modified human globin for content of "essential" amino acids

	Analysis of globin* *Gm./100 Gm.*	"Minimum daily requirement" (13) *Gm.*
Methionine	1.0	1.10
Tryptophan	1.5	0.25
Isoleucine	0.3	0.70
Leucine	12.9	1.10
Valine	9.2	0.80
Histidine	5.6	†
Arginine	2.9	†
Threonine	3.5	0.50
Phenylalanine	5.7	1.10
Lysine	8.5	0.80

* The amino acids indicated, excepting tryptophan, were determined by microbiologic assay (14) of lot number 38–2 of modified human globin following acid hydrolysis. Tryptophan was determined microbiologically following alkaline hydrolysis of the globin. The manufacturer's analysis of other lots of globin indicate an isoleucine content of 0.6 per cent and Albanese reported human hemoglobin to contain 0.5 per cent of this amino acid (15).

† Not required for maintenance of nitrogen equilibrium in adult man (13).

[4] This diet maintained nitrogen balance and body weight in a normal male when casein was provided as the sole source of dietary protein.

[5] "Dayamin," furnished by Abbott Laboratories, North Chicago, Illinois, and containing per capsule: thiamine hydrochloride 5 mg., riboflavin 5 mg., ascorbic acid 100 mg., nicotinamide 25 mg., pantothenic acid 5 mg., pyridoxine 1.5 mg., vitamin A 10,000 U.S.P. units, and vitamin D 1,000 U.S.P. units.

[6] Provided by Merck and Company, Rahway, New Jersey.

days. Were isoleucine a limiting amino acid, an immediate negative nitrogen balance with increase in the output of urinary nitrogen would be expected. This did not occur. In fact, the nitrogen balance and body weight during the period in which globin was administered alone by mouth was comparable to the previous period in which isoleucine was given in addition to globin. During the ensuing nine days of study the patient was given food protein containing nitrogen approximately equivalent to that in the previously administered globin. There was essentially no change in nitrogen balance. A second normal individual (J. G., Figure 2) studied in a similar manner yielded comparable results.

A third patient (J. B., Figure 3) was provided with a similar protein-free basal diet and given globin equivalent to 7.1 Gm. of nitrogen intravenously daily as the sole source of protein for five days. Nitrogen balance and body weight were maintained. The trend toward less positive nitrogen balance during the last two days of this period of study was due to an increase in the urinary excretion of protein. Earlier in his study, J. B. was given globin intravenously in addition to a 50 Gm. protein diet. During the period of globin administration nitrogen balance was more positive than during the initial control period, indicating retention of globin nitrogen.

F. O'D. (Figure 4) illustrates another metabolic study in which globin was administered intravenously as a supplement to a diet. During the initial six day control period, the patient was maintained on a constant diet providing 35 Gm. of protein and 3,000 calories daily. Globin providing 4.8 Gm. of nitrogen was added intravenously daily during the next six days. This regimen was followed by a second six-day control period. During the period of intravenous globin administration, a more positive nitrogen balance was obtained than during either the fore- or after-control period. Urine nitrogen excretion increased significantly during globin administration, but was largely attributable to protein (presumably globin) in the urine. In the control period following intravenous globin administration there was an increased output of urinary non-protein nitrogen and a slight negative nitrogen balance suggesting metabolism of the previously administered protein. During the last nine-day period of study this patient was given a quantity of dietary protein equivalent in terms of nitrogen content to the globin previously

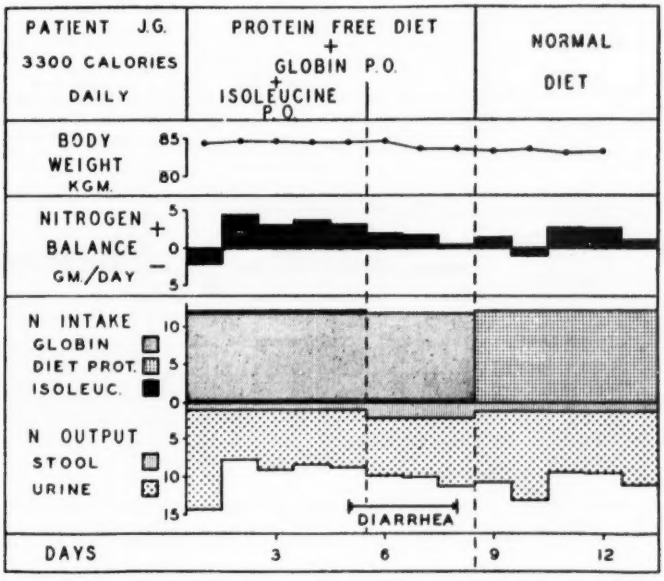

FIG. 2. MODIFIED HUMAN GLOBIN ADMINISTERED ORALLY AS A SOLE SOURCE OF PROTEIN WITH AND WITHOUT ISOLEUCINE

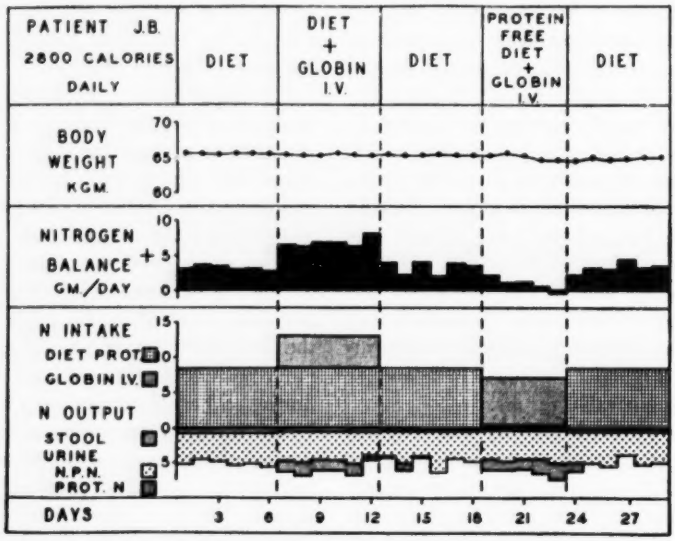

FIG. 3. MODIFIED HUMAN GLOBIN ADMINISTERED INTRAVENOUSLY AS A SUPPLEMENT TO A DIET AND THEN AS A SOLE SOURCE OF PROTEIN

administered intravenously. Positive nitrogen balance again resulted, and was comparable in magnitude to the balance obtained during the period of globin infusions. A similar study was done in another patient, J. R., (Figure 5). The results of this study were comparable to those ob-

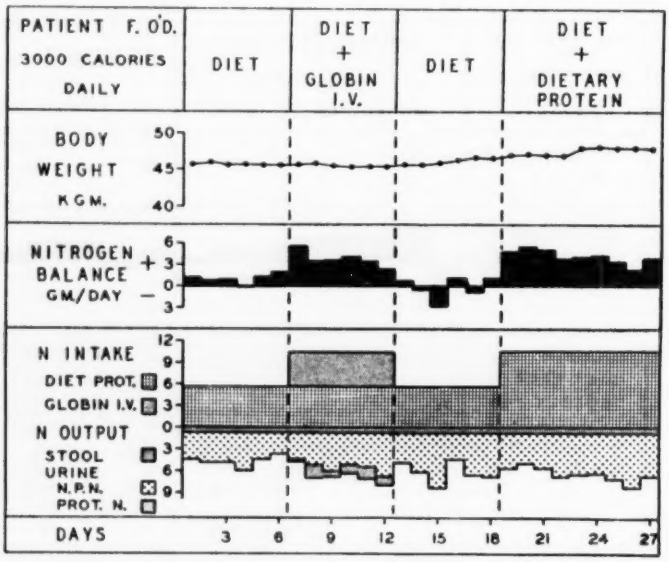

FIG. 4. MODIFIED HUMAN GLOBIN ADMINISTERED INTRAVENOUSLY AS A SUPPLEMENT TO A DIET AND COMPARED TO AN EQUIVALENT QUANTITY OF DIETARY PROTEIN

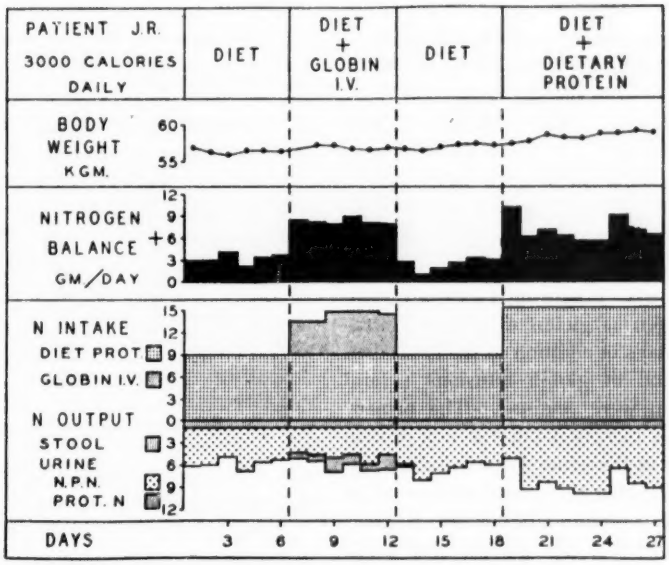

FIG. 5. MODIFIED HUMAN GLOBIN ADMINISTERED INTRAVENOUSLY AS A SUPPLEMENT TO A DIET AND COMPARED TO AN EQUIVALENT QUANTITY OF DIETARY PROTEIN

served in F. O'D., intravenously administered globin again comparing favorably with an approximately equivalent amount of dietary protein.

The metabolic data of another subject, W. O., maintained on a constant diet providing 50 Gm. of dietary protein and 2,800 calories per day are

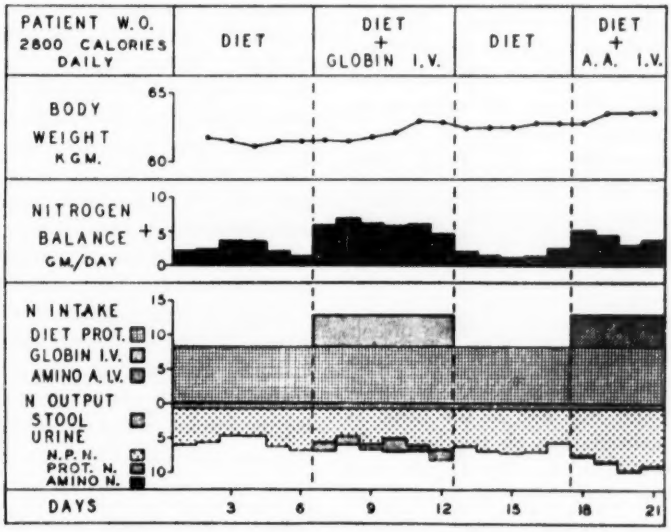

FIG. 6. MODIFIED HUMAN GLOBIN ADMINISTERED INTRAVENOUSLY AS A SUPPLEMENT TO A DIET AND COMPARED TO AN EQUIVALENT QUANTITY OF AMINO ACIDS GIVEN INTRAVENOUSLY

TABLE II

Proteinuria following intravenous infusions of globin

Patient	Globin *Gm./day*	Days	Average infusion time *Hours*	Urinary protein *Gm./day*	Per cent excreted
J. R.	32	6	3	8.0	25.0
W. O.	32	6	2¼	7.2	22.5
F. O'D.	32	6	2	7.2	22.5
J. B.	48	5	2	11.3	23.5
J. B.	32	6	1½	9.2	28.8

presented in Figure 6. Following a control period, 4.6 Gm. of nitrogen as globin were given intravenously for six days in addition to the basal diet. During this period of globin infusions several grams of nitrogen were retained. The urinary nitrogen excretion increased but the non-protein nitrogen excreted in the urine did not change significantly indicating that this increase was due to the excretion of protein. Following a post-infusion control period, the patient was given intravenously a solution of amino acids [7] containing ample quantities of the essential amino acids. This solution furnished an equivalent amount of nitrogen, and was given in the same volume of fluid and at the same rate of infusion as the previously administered globin. Positive nitrogen balance again occurred during a four-day period of observation, but was less than that observed during globin administration. This less positive nitrogen balance could not be attributed to aminoaciduria, since only a small proportion of the infused amino acids were lost in the urine.

The increased urinary excretion of nitrogen noted in the patients during intravenous globin administration was due to proteinuria primarily. Table II indicates the extent of this loss in the patients studied, to whom 32 to 48 Gm. of globin were given intravenously daily for five to six days with average infusion times ranging from one and one half to three hours. The daily loss of protein in the urine ranged from 7.2 to 11.3 Gm. Thus, 22.5 to 28.8 per cent or approximately one-fourth of the infused globin was lost in the urine as protein. The proteinuria had no relation to the time required for the infusion. The magnitude of proteinuria following globin infusions noted in the present study is similar to that reported by Waterhouse, Fenninger, and Keutmann (5), but greater than that observed by others (7, 16).

[7] "Solution of Amino Acids" furnished by Merck and Company, Rahway, New Jersey.

TABLE III

Proteinuria (Gm. per day) following I.V. human globin and albumin

Days	Days of infusion: Patient J. R. 32 Gm. globin I.V. daily	Days of infusion: Patient C. J. 50 Gm. albumin I.V. daily	Days after infusion: J. R.	Days after infusion: C. J.
1	5.6	0.0	2.5	6.3
2	5.6	0.0	0.0	7.7
3	12.5	0.0	0.0	5.0
4	8.1	0.0	0.0	3.5
5	5.6	0.0	0.0	1.7
6	10.6	1.0	0.0	0.5
7		1.0	0.0	0.3
8		2.2	0.0	0.0
9		5.0	0.0	0.0
16		8.0		

Table III contrasts modified human globin and concentrated human serum albumin with respect to the proteinuria which occurs following their intravenous administration. Patient J. R. received 32 Gm. of globin daily for six days. On the first day and on each successive day of globin administration, significant quantities of protein appeared in the urine; but proteinuria was not present during the days immediately following cessation of globin infusions. Patient C. J. received 50 Gm. of albumin intravenously daily for 16 days. Proteinuria did not occur until the sixth day of infusion and then only gradually increased. After discontinuation of albumin therapy, the proteinuria cleared gradually during the next six days. This disparity in excretion rates may be related to the difference in molecular size of the two protein preparations since the molecular weight of albumin is approximately twice that of the modified human globin preparation used.

Modified human globin was not detected in the serum 24 hours following single or multiple infusions. Moreover, in one study electrophoretic analysis [8] of the serum obtained 24 hours following the administration of 192 Gm. of globin in six days did not show the characteristic boundary of

[8] The analysis was done by Dr. Peter Bernfeld, Tufts College Medical School, using veronal buffer pH 8.60, ionic strength u 0.1, electrical field 8.8 V./cm. The photographs were taken after 4,800 seconds. The mobility of the globin was 4.28×10^{-5} cm.^{2} volt^{-1} seconds^{-1}.

globin. The rapid disappearance of infused globin from the blood has been noted by others (7, 16). In contrast to this, following the daily administration of concentrated human serum albumin the level of albumin in the blood progressively rises and after cessation of administration there follows a slow and progressive decrease (17). The complete removal of globin from the blood even when given on consecutive days probably indicates that, apart from the fraction excreted in the urine, it is metabolized or stored in the body. The rise in plasma and urine alpha amino nitrogen which occurred following the infusion of globin (Figure 7) offers further evidence that the globin is metabolized. These increases are of such magnitude that they cannot be accounted for by the free alpha amino nitrogen content of the globin itself.

II. *Clinical tolerance.* Fifty-two infusions of freshly mixed 4 per cent globin in water were administered to twenty individuals in quantities ranging from 16 to 32 grams per infusion and at rates varying from 0.14 to 0.96 gram per kilogram per hour. Four subjects received 32 grams intravenously daily for six consecutive days.

There were no serious untoward reactions, although the incidence of annoying reactions was high (Table IV). The most frequent of these was flushing which occurred with most of the lots of globin used. The intensity of the erythema was proportional to the speed of the infusion and could be increased or decreased by respectively altering the infusion rate. The second most common undesirable effect was pruritis which appeared in many of the patients displaying the flush. Mild

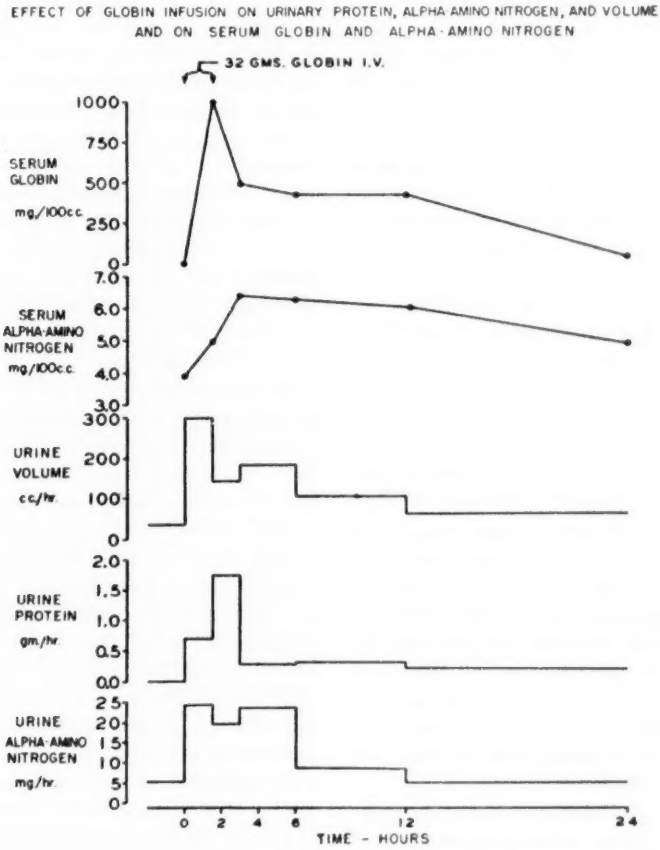

FIGURE 7

TABLE IV

Clinical tolerance to modified human globin 52 infusions to 20 subjects*

Reactions	No. reactions	No. subjects	Per cent infusions	Per cent subjects
Flush:				
Marked	8	12	67	60
Moderate	18			
Mild	9			
Total	35			
Itching	21	9	40	45
Chills and fever	6	6	12	30
Nausea	1	1	2	5
Anorexia	0			
Vomiting	0			
Local reaction	0			

Blood pressure, pulse, respiration: No consistent or significant changes.

* Each infusion furnished 16 or 32 Gm. of globin.

pyrogenic reactions also occurred, but were limited to four of the twelve lots of globin used. Local reactions did not occur. Blood pressure, pulse, and respiratory rates taken before and after 20 infusions in 14 subjects showed no noteworthy changes. Despite eleven infusions of a total of 432 Gm. of globin administered over the course of 17 days to one subject (J. B.) with minimal cirrhosis of the liver by biopsy, the bromsulphalein retention test and the serum bilirubin level remained normal.

Complete urinalyses were done immediately before and after globin infusions in 11 of these patients. No abnormalities developed in tests for albumin (heat and acetic acid), for reducing substances (Benedict's reagent), or in microscopic examination of the sediments. All of the post-infusion urines, however, produced a heavy precipitate with Esbach's reagent (a solution of picric and citric acids). A solution of 4 per cent globin, similarly tested, likewise gave a heavy precipitate with Esbach's reagent but no precipitate with heat and acetic acid, suggesting that the proteinuria accompanying the globin infusions resulted from the renal excretion of the infused material. These two tests for urinary protein offered a simple means of distinguishing "globinuria" from the usual proteinuria, as the latter is precipitated by heat and acetic acid as well as by Esbach's reagent.

To investigate the possibility of *in vivo* hemolysis with 4 per cent globin, urine guaiac tests were made on the eleven pre- and post-infusion urines. All pre-infusion specimens were guaiac negative, while eight of the eleven post-infusion specimens gave a positive reaction. But since the 4 per cent aqueous solution of globin itself was found to give a markedly positive guaiac (and benzidine) test, the guaiac positivity of the post-infusion urines was due presumably to the urinary excretion of the infused material. "Red urine" was not observed in any of the patients who received globin intravenously.

To determine whether or not globin interferes with the typing and cross-matching of blood, two patients were infused with 32 grams of globin in one hour. Blood samples drawn immediately after the infusion showed no rouleaux formation, and typing and cross-matching were accomplished without difficulty.

COMMENT

Solutions of protein for parenteral feeding should be free from untoward reactions, and the protein should be of good quality and readily available to the body cells. The availability of globin is indicated by its ability to maintain nitrogen balance when given as a sole source of nitrogen orally, by its approximate equivalence nutritionally to comparable quantities of nitrogen as protein by mouth or amino acids intravenously, and by the failure to find globin in the blood 24 hours after prolonged daily parenteral administration. Finally, the rapid appearance of aminoacidemia and aminoaciduria following globin infusions suggest a rapid breakdown of the globin. Human serum albumin given intravenously has a 50 per cent disappearance time of about five to ten days (17, 18). In contrast, the half-life of globin is probably several hours as indicated in the present study and studies utilizing I^{131} tagged globin (16). Untoward reactions following globin infusions, although frequent in occurrence, were never severe nor harmful in the studies reported here. Their incidence has been reported to have decreased with lots of globin manufactured more recently than those used in this study (6). The urinary excretion of large quantities of protein following the intravenous administration of globin gives rise to a consideration of possible renal damage. The suggestion that some of the urinary protein is not globin but serum protein (6) supports this thesis, as do occasional reports of hematuria. Further

and more detailed studies of renal function must be done to exclude renal damage as a complication of globin administration. Post-infusion urine examinations were not abnormal in the present study and Brandt, Caccese, and Ginsberg (19) found no evidence of changes in renal function following globin administration.

Considering the high level of urinary excretion, and rapid breakdown in the body of globin as compared to albumin, one might suspect that globin would be a less effective plasma expander than albumin or plasma. Nevertheless, the duration of action of globin may be sufficient to warrant its use as a plasma volume expander in the management of emergency situations. For example, globin has been used in the treatment of postoperative shock and shock accompanying hemorrhage or burns (*2, 6, 7*).

Although the data presented are too limited to justify a comparison of the nutritive adequacy of globin to solutions of amino acids or protein hydrolysates, the usefulness of parenterally administered globin may lie in its ability to furnish available protein.

SUMMARY AND CONCLUSIONS

The nutritive value of modified human globin was investigated by nitrogen balance studies in six men. The studies indicate that nitrogen balance and body weight were maintained while globin was given orally to two men for 8 and 11 days, respectively, as the sole source of protein in a calorically adequate diet. Although the quantities of globin administered orally provided less than the tentative minimum daily requirement of isoleucine as estimated by Rose (13), the oral supplementation of globin with this amino acid did not enhance the nutritive adequacy of globin as measured by nitrogen balance in two subjects. Globin given intravenously as a supplement to a constant diet resulted in increased positive nitrogen balance and retention of nitrogen of magnitude comparable to that observed during subsequent study periods during which equivalent quantities of dietary protein were ingested. The increases in urinary nitrogen excretion noted during intravenous globin administration were attributable primarily to the excretion of approximately 25 per cent of the infused material as protein.

That infused globin rapidly disappears from the blood is indicated by the prompt cessation of proteinuria after globin infusions, and the failure to detect significant quantities of globin in the serum 24 hours after its administration. The rise in plasma and urinary alpha amino nitrogen which occurred following the infusion of this protein suggest it is rapidly metabolized.

No serious untoward reactions resulted from intravenously administered modified human globin, although transient flushing, pruritus, and mild pyrogenic reactions occurred frequently.

These studies indicate that modified human globin may be useful as a source of protein for parenteral alimentation. Investigation of its adequacy as a plasma volume expander was not undertaken, but the prompt disappearance from the blood of intravenously administered globin, resulting, in part at least, from its rapid rate of metabolism and high level of urinary excretion, suggests that its usefulness for this purpose may be limited.

ACKNOWLEDGMENTS

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PULMONARY FUNCTION IN BOECK'S SARCOID

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Boeck's sarcoid frequently produces widespread roentgenologic evidence of pulmonary disease without clinical signs or symptoms. Wright and Filley (1) reported normal pulmonary function studies in one such case. Others (2, 3, 4, 5) have reported a variety of effects of more advanced sarcoidosis on pulmonary function. This report is a study of pulmonary function in six cases of Boeck's sarcoid and emphasizes the frequent coincidence of widespread disease without severe functional impairment.

MATERIALS AND METHODS

The six patients included in this report were all young males in Walter Reed Army Hospital. In each instance a positive diagnosis of Boeck's sarcoid was made on histologic material (peripheral lymph node or lung [Case I]), and in each instance exhaustive study ruled out the presence of tuberculosis. The pertinent clinical data are summarized in Table I.

Maximum breathing capacity was determined by having the subject breathe as deeply and as rapidly as possible for 15 seconds through a low-resistance respiratory valve into a Douglas bag. The predicted normal value is based on a formula by Wright (6) which obtains for normal males studied in this laboratory.

Pulmonary ventilation-perfusion studies were performed as outlined by Riley, Cournand, and Donald (7). Fasting subjects lay supine for twenty minutes breathing room air with an indwelling needle in the radial artery, whereupon expired air was collected over a three-minute interval and arterial blood was allowed to flow into a syringe the dead space of which was filled with mercury and a heparin-fluoride mixture. The arterial sample was collected for a 1-minute interval during the middle of the expired air collection and was analyzed immediately for blood gas tensions. Subsequently, the study was repeated during the inhalation of 14 per cent oxygen after a 20-minute interval had elapsed in order to achieve a steady state.

Gas volumes were measured in a Tissot spirometer and corrected to body temperature. Gas composition was measured by the Scholander micro-gas analyzer (8) and duplicates checked within plus or minus 0.03 volume per cent. The RQ of the expired air was calculated from the alveolar equation (9). Arterial pCO_2 and pO_2 were measured by Riley's technique (10) and all duplicate analyses checked within plus or minus 3 mm. Hg. In standardizing this technique a large number of analyses was performed on samples of blood previously equilibrated in a tonometer with gas of known tensions. Agreement was good between blood and gas tensions and there was no need for the correction factors recommended by Riley.

The effective alveolar oxygen tension was calculated from the alveolar equation, substituting arterial pCO_2 for alveolar pCO_2. From a knowledge of the alveolar-arterial oxygen gradient at two levels of oxygenation, the effects of venous admixture and of impaired diffusion on reducing the arterial oxygen tension can be calculated (11, 12). The per cent venous admixture and the oxygen diffusing capacity were calculated from this data by means of charts developed by Riley, Cournand, and Donald (7).

TABLE I

Clinical data on six patients with Boeck's Sarcoid

Case	B.S.A.	Age	X-ray	Symptoms
I	1.85	23	Large, rounded, discrete lesions throughout both lung fields.	Spontaneous pneumothorax on left. No other cardiorespiratory symptoms.
II	1.75	24	Slight hilar adenopathy; generalized hazy infiltration through both lungs.	No cardiorespiratory symptoms. 5-month history of uveitis due to sarcoidosis.
III	1.88	26	Slight hilar adenopathy; generalized hazy infiltration through both lung fields.	9-month history of vague chest pain without cough or dyspnea.
IV	1.70	26	Moderate hilar adenopathy; increased lung markings with slight nodularity.	1-year history of intermittent dry cough and shortness of breath on heavy exertion. Twice treated with cortisone.
V	1.80	22	Marked hilar adenopathy and hazy parenchymal infiltration.	1-year history of progressive exertional dyspnea; 4 months of dry cough.
VI	1.92	26	Hilar adenopathy.	No cardiorespiratory symptoms. Diagnosis made on routine chest X-ray 6 months before study.

Accurate calculation of the oxygen diffusing capacity requires that the effective alveolar-effective capillary oxygen tension gradient be at least 4 mm. Hg. In a study of nine normal individuals in this laboratory a mean diffusion gradient of 1 mm. Hg was found so that the normal diffusing capacity cannot be calculated. A value of 20 has been arbitrarily selected as the low limit of normal for DO_2. Twenty-one normal distribution gradients were measured in this laboratory and the mean gradient was 9 mm. Hg, corresponding to a venous admixture effect of 4 per cent. Similar values for the normal distribution gradient have been reported by Ryan and Hickam (13), Filley, Gregoire, and Wright (14), and Blount, McCord, and Anderson (15). However, Comroe and Dripps (16) and Galdston and Wollack (17) have found no difference between effective alveolar oxygen tension and arterial oxygen tension in normal subjects. In dogs (18) the size of the distribution gradient has been found to vary directly with the magnitude of the alveolar ventilation, and a similar relationship has been found in man (19). This effect is probably due to uneven ventilation which causes different RQs in various parts of the lung and, hence, a gradient between effective alveolar air and arterial blood (18, 20). Thus, the venous admixture effect may vary from 0 to 8 per cent in normal individuals depending upon the nature of the ventilation.

The dead space was calculated from the Bohr equation (9) and, in normal people, occupies less than 30 per cent of the tidal volume. The relative size of the dead space is also dependent upon the nature of the ventilation and may be increased up to 40 per cent of the tidal volume if the breathing is rapid.

RESULTS

The results of these studies are summarized in Tables II and III. Spirometry was performed on all patients, and in none was there evidence of respiratory obstruction.

Case I was entirely asymptomatic. Six months before the time of the study he had a spontaneous pneumothorax, following which chest X-ray showed extensive parenchymal involvement with many large rounded densities scattered through both lung fields. Five months before study lung biopsy revealed granulomatous disease consistent with Boeck's sarcoid. Chest X-ray was unchanged at the time pulmonary function studies were performed and the studies were entirely normal.

Case II had no cardiorespiratory symptoms, although X-ray revealed moderate hilar adenopathy and slight generalized parenchymal infiltration. The venous admixture effect was at the upper limits of normal, and other studies were entirely normal.

Case III reported no cardiorespiratory symptoms other than a nine-month history of vague chest pain. X-ray revealed slight hilar adenopathy and a diffuse, hazy parenchymal infiltration. Ventilatory function was normal, but the patient exhibited hyperventilation at rest. Since this was

TABLE II

Gas exchange data

Case	Inspired oxygen tension $P_{I_{O_2}}$ *mm. Hg*	"Effective" alveolar oxygen tension $P^*_{A_{O_2}}$ *mm. Hg*	Art. P_{O_2} $P_{a_{O_2}}$ *mm. Hg*	Art. P_{CO_2} $P_{a_{CO_2}}$ *mm. Hg*	Expired P_{CO_2} $P_{E_{CO_2}}$ *mm. Hg*	Ventilation *L./min.* BTPS $\dot{V}_E$	Resp. rate f	Oxygen consumption *ml./min.* STPD $\dot{V}_{O_2}$	Respiratory quotient R
I	148	93	88	41	30	2.62	7	243	.70
	100	56	55	39	28	3.48	10	236	.87
II	148	99	88	38	26	4.35	14	305	.74
	96	52	48	37	26	4.25	12	280	.81
III	148	94	87	41	20	4.87	21	293	.71
	96	54	44	38	21	5.07	16	262	.90
IV	148	98	87	39	28	2.92	8	218	.73
	101	60	55	36	27	3.17	10	199	.86
	150	96	83	38	25	3.07	8	232	.66
	102	62	55	33	17	4.81	11	198	.81
V	150	102	77	34	13	6.72	40	281	.65
	98	60	48	34	15	6.89	34	253	.87
VI	149	93	89	39	20	3.95	17	263	.65
	101	58	53	38	22	4.38	19	254	.86

TABLE III

Summary of ventilation, distribution, and diffusion data

Case	Maximum breathing capacity *L./min.* BTPS		Vital capacity *Liters* BTPS		Ratio of dead space to tidal volume	Ratio of venous admixture to total blood flow	Oxygen diffusing capacity of the lungs *cc./min./mm. Hg*
	Obs.	Pred.	Obs.	Pred.	$V_D/V_T \times 100$	$Qva/Qt \times 100$*	DO_2
I	185	185	5.0	4.6	22	3	Normal†
II	179	184	5.6	4.4	21	6	Normal
III	168	181	3.4	4.7	37	4	15
IV	136	181	4.0	4.3	18	6	Normal†
	136	181	3.8	4.3	25	8	14
V	138	184	3.0	4.5	40	14	15
VI	139	181	5.4	4.8	28	2	18

* Calculations are made with an assumed A-V difference of 25 per cent saturation.

† If the $P^*A_{O_2} - Pa_{O_2}$ gradient is less than 4 mm. Hg during 14 per cent O_2 breathing DO_2 is represented simply as "Normal."

not present on exercise, it probably was of functional origin rather than due to the pulmonary disease. This patient showed a slightly diminished diffusing capacity and a large dead space without abnormal venous admixture. In the absence of seriously impaired diffusion or blood-gas distribution the increase in relative dead space may be attributed to the hyperventilation.

Case IV gave a one-year history of intermittent cough and dyspnea on heavy exertion for which he had twice received five-week courses of cortisone therapy, one year and nine months before studied. X-ray revealed hilar adenopathy and increased lung markings with nodularity. The data obtained on two occasions, separated by a ten-day interval, are both included in the tables. Maximum breathing capacity was reduced, although vital capacity was normal. The dead space was not increased but the venous admixture effect was at the upper limit of normal and the diffusing capacity at the lower limit of normal.

Case V gave a one-year history of exertional dyspnea which was accompanied more recently by a slightly productive cough. X-ray revealed marked hilar adenopathy and generalized hazy infiltrations of the lung parenchyma. He showed abnormality in all functions measured with reduced MBC and vital capacity, hyperventilation at rest and on exercise, diminished diffusing capacity, a large venous admixture effect and a large dead space.

Case VI had no cardiorespiratory symptoms. Six months before the study, routine chest X-ray showed hilar adenopathy with some nodular increase in lung markings in the perihilar areas. Although his vital capacity was normal, he showed a diminished maximum breathing capacity. Nevertheless, there was no increase in dead space or venous admixture, and diffusing capacity was normal.

DISCUSSION

The abnormalities in pulmonary function in Boeck's sarcoid exhibit great variability. This might be expected from a knowledge of the pathology of the disease. Basically, the lesions consist of granulomata in the alveolar walls with large areas of normal lung interspersed between the diseased tissues, and accumulations of granulomatous lesions in the peribronchial and perivascular areas (21). These granulomata generally become fibrosed, and the extent of the resultant scar tissue determines the effect of the disease on lung function. Patients with peripheral granulomata without scarring and with large areas of normal lung (Case I) may exhibit perfectly normal lung function at rest. Another such case has been reported by Wright and Filley (1), and the only abnormality in that individual was hyperventilation on exercise.

On *a priori* grounds, one would anticipate that extensive scarring of the lung parenchyma would lead to impairment of ventilatory function, hyperventilation at rest, and an increase in the size of the lung dead space. One or more of these findings was evident to a slight extent in Cases III, IV, and VI. In these cases the process had not progressed to the point of significant interference

with the diffusion of oxygen or with the ventilation-perfusion relationship of sufficient area of lung to cause an increase in venous admixture. Similar findings have been observed by others in one patient who had diminished ventilatory function (MBC and vital capacity) and an increased lung dead space but normal resting diffusing capacity and venous admixture (*22*). Baldwin, Cournand and Richards (5) have included five such cases of Boeck's sarcoid in their group 1 of pulmonary fibrosis.

More extensive involvement of the lungs with the pathologic process leads to generalized pulmonary fibrosis with interference of all phases of pulmonary function. Case V is an example of generalized pulmonary fibrosis with evidence of alveolar-capillary block. In addition to impaired ventilatory function (hyperpnea at rest) and a large dead space, he had a greatly increased venous admixture effect and slightly diminished oxygen diffusing capacity. Reports by others (3, 4) include five more such cases with the manifestations of "alveolar-capillary block."

The presence of granulomata in the hilar lymph nodes and peribronchial areas exerts no effect on pulmonary function unless there is encroachment on the airways. None of these patients demonstrated signs of obstructive disease, although Coates and Comroe (*2*) reported five out of eight cases of Boeck's sarcoid who showed this type of abnormality.

Thus, the physiologic abnormalities in Boeck's sarcoid would appear to be quite variable but dependent upon the location and the extent of the pathologic process. Peripheral granulomata, even though extensive, may exert no measurable effect on pulmonary function at rest. Accumulations of fibrous tissue around the airways may lead to a predominantly obstructive type of disease. Extensive scarring of the parenchyma may lead to restriction of lung volume, impairment of maximum ventilatory function, increase in the size of the pulmonary dead space, and an augmented venous admixture effect, probably due to uneven ventilation and perfusion of the lung. Finally, diffuse fibrosis may cause impairment of the diffusing capacity of the lungs, either by thickening of the alveolar wall or by reduction in the number of pulmonary capillaries, as discussed by Riley, Riley, and Hill (4). Such effects may occur singly, but in advanced cases they generally occur in combination. In the cases herein reported, no particular combination of defects was obvious, three patients having a single different abnormality. The diffusing capacity in these patients was only measured at rest, and it is quite possible that their maximum diffusing capacities were subnormal, in view of the apparent frequency of "alveolar-capillary" block in this disease. Only two patients experienced exertional dyspnea and in all but Case V the degree of functional impairment was slight despite the presence of large areas of disease evidenced by X-ray.

Finally, a word should be said about therapy with cortisone or ACTH. Although the functional deficit may correlate with the patient's symptoms, one cannot correlate the extent of disease demonstrable by X-ray with the effect of the disease on pulmonary function. Since pulmonary tuberculosis may easily be mistaken for Boeck's sarcoid, and since cortisone and ACTH are considered to exert an unfavorable action on tuberculosis, such therapy should not be considered on the basis of the amount of disease which is demonstrated by X-ray alone. Cortisone and ACTH should be reserved for those cases in which there is evident effect of the disease on pulmonary function.

SUMMARY

Observations are presented of some pulmonary functions in six patients with Boeck's sarcoid. There was little correlation between the degree of roentgenologic involvement and of impaired pulmonary function. Pulmonary function in such patients at rest may be normal despite the presence of large areas of diseased lung by X-ray. Sarcoidosis may cause pulmonary fibrosis with diminished ventilatory function and increased pulmonary dead space. In advanced cases, these abnormalities may be accompanied by impairment of oxygen diffusing capacity and of pulmonary ventilation-perfusion relationships.

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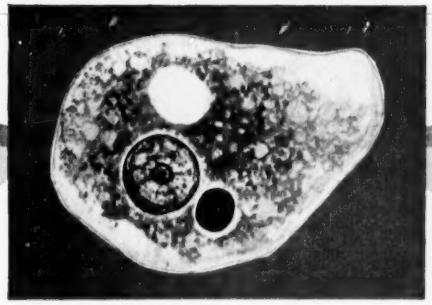

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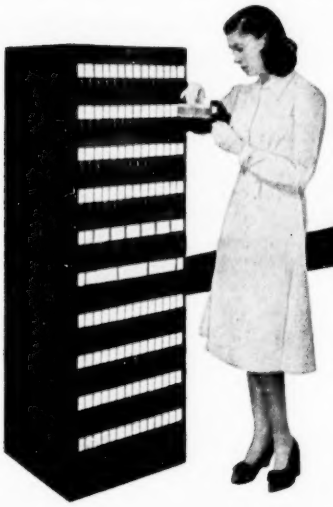

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